(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 July 2003 (03.07.2003)

PCT

(10) International Publication Number
WO 03/053407 A1

(51) International Patent Classification[7]: A61K 9/127, 9/50, 9/51

(21) International Application Number: PCT/US02/40846

(22) International Filing Date:
19 December 2002 (19.12.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/342,156	19 December 2001 (19.12.2001)	US
60/406,807	29 August 2002 (29.08.2002)	US
60/418,602	15 October 2002 (15.10.2002)	US

(71) Applicant: **RESEARCH DEVELOPMENT FOUNDATION** [US/US]; 402 North Division Street, Carson City, NV 89703 (US).

(72) Inventors: **SANDERS, Bob, G.**; 2005 Barton Hills Drive, Austin, TX 78720 (US). **KLINE, Kimberly**; 2505 McCallum, Austin, TX 78703 (US). **LAWSON, Karla, A.**; 203 East 31st Street #309, Austin, TX 78705 (US). **MENCHACA, Marla, S.**; 11304 Tanya Trail, Austin, TX 78726 (US). **KNIGHT, J., Vernon**; 29 Lana Lane, Houston, TX 77027 (US). **WELLEN, Clyde, W.**; 2121 Kirby #79, Houston, TX 77019 (US).

(74) Agent: **ADLER, Benjamin, A.**; Adler & Associates, 8011 Candle Lane, Houston, TX 77071 (US).

(81) Designated States *(national)*: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW.

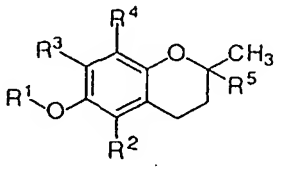
(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/053407 A1

(54) Title: LIPOSOMAL DELIVERY OF VITAMIN E BASED COMPOUNDS



(I)

(57) Abstract: The present invention provides a method for treating a cell proliferative disease by delivering a composition comprising a vitamin E based anti-cancer compound contained within a delivery vesicle of an individual in need of such treatment where the compound has a structural formula where R' is a hydrogen or a carboxylic acid; R^2 and R^3 are hydrogen or R^4; R^4 is methyl; and R^5 is alkyl. Also provided is a vesicle comprising these compounds.

LIPOSOMAL DELIVERY OF VITAMIN E BASED COMPOUNDS

BACKGROUND OF THE INVENTION

Cross-reference to Related Application

This non-provisional patent application claims benefit of provisional patent applications United States Serial No. 60/416,602, filed October 15, 2002, now abandoned, United States Serial No. 60/406,807, filed August 29, 2002, now abandoned and United States Serial No. 60/342,156, filed December 19, 2001, now abandoned.

Field of the Invention

This invention relates to the fields of pharmacology and cancer treatment. More specifically, this invention relates to delivery of liposomal preparations of vitamin E based compounds as an effective method for the treatment and the prevention of cancer.

Description of the Related Art

Regulatory controls of pro-life (survival) and pro-death (apoptosis) are extremely complex, involving multiple intracellular signaling pathways and multiple interacting gene products. Cancer cells may exhibit enhanced expression of genes and their products that promote cell proliferation, allowing cancer cells to increase in number. In addition to enhanced expression of pro-life genes, cancer cells down-regulate genes and their products that control pro-death signals, resulting in the accumulation and enhanced metastasis of life threatening cancer cells. Combinations of unregulated cell proliferation and suppression of death inducing signaling pathways give cancer cells both growth and survival advantages.

Whether a cell increases in numbers or not depends on a balance of expression of negatively-acting and positively-acting growth regulatory gene products, and the presence or absence of functional cell death signaling pathways. Negative-acting growth regulatory genes contribute to blockage of cells in the cell cycle. Positive-acting growth regulatory genes stimulate cells to progress through the cell cycle. Genes involved in apoptosis can be either proapoptotic or antiapoptotic, and the dynamic balance between them determines whether a cell lives or dies.

A wide variety of pathological cell proliferative conditions exist for which novel therapeutic strategies and agents are needed to provide benefits. These pathological conditions may occur in almost all cell types capable of abnormal cell proliferation or abnormal responsiveness to cell death signals. Among the cell

types that exhibit pathological or abnormal growth and death characteristics are fibroblasts, vascular endothelial cells, and epithelial cells. Thus, novel methods are needed to treat local or disseminated pathological conditions in all or almost all organ and tissue systems of individuals.

Most cancers, whether they be male specific such as prostate or testicular, or female specific such as breast, ovarian or cervical or whether they affect males and females equally such as liver, skin or lung, with time undergo increased genetic lesions and epigenetic events, and eventually may become highly metastatic and difficult to treat. Surgical removal of localized cancers has proven effective only when the cancer has not spread beyond the primary lesion. Once the cancer has spread to other tissues and organs, the surgical procedures must be supplemented with other more specific procedures to eradicate the diseased or malignant cells. Most of the commonly utilized supplementary procedures for treating diseased or malignant cells such as chemotherapy or radiation are not localized to the tumor cells and, although they have a proportionally greater destructive effect on malignant cells, often affect normal cells to some extent.

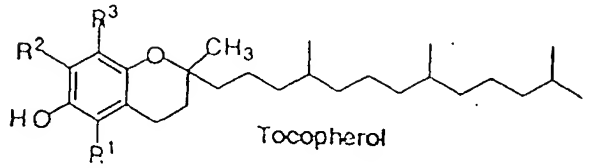
Some natural vitamin E compounds, and some derivatives of vitamin E have been used as proapoptotic and DNA synthesis inhibiting agents and thereby potent anti-cancer agents. Structurally, vitamin E is composed of a chromanol head and an alkyl side chain. There are eight major naturally occurring forms of vitamin E: alpha (α), beta (β), gamma (γ), and delta (δ) tocopherols and α, β, γ, and δ tocotrienols. Tocopherols differ from tocotrienols in that they have a saturated phytyl side chain rather

than an unsaturated isoprenyl side chain. The four forms of tocopherols and tocotrienols differ in the number of methyl groups on the chromanol head (α has three, β and γ have two and δ has one) as shown in Table 1.

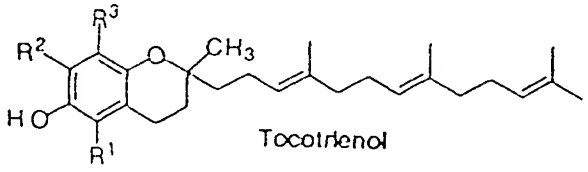
TABLE 1

General structure of tocopherols and tocotrienols

	R^1	R^2	R^3
Alpha (α)	CH_3	CH_3	CH_3
Beta (β)	CH_3	H	CH_3
Gamma (γ)	H	CH_3	CH_3
Delta (δ)	H	H	CH_3



Tocopherol



Tocotrienol

Several studies have described potent anti-tumor activity of RRR-α-tocopheryl succinate (vitamin E succinate; VES), a hydrolyzable ester derivative of RRR-α-tocopherol. Prasad and Edwards-Prasad were the first to describe the capacity of vitamin E succinate but not other forms of vitamin E to induce morphological alterations and growth inhibition of mouse melanoma B-16 cells and to suggest that vitamin E succinate might be a useful tumor therapeutic agent (1). Additional studies have demonstrated that vitamin E succinate is a potent growth inhibitor of a wide variety of epithelial cancer cell types, including breast, prostate, lung, and colon; as well as, hematopoietic-lymphoid leukemia and lymphoma cells, *in vitro* (2-7).

Recent studies have demonstrated vitamin E succinate to have anti-tumor activity in animal xenograft and allograft models when administered intraperitoneally (i.p.)(8-11), suggesting a possible therapeutic potential. Vitamin E succinate administered i.p. or orally (p.o.) has also been shown to have inhibitory effects on carcinogen [benzo(a)pyrene]-induced forestomach carcinogenesis in mice, suggesting potential as an anti-carcinogenic agent (12). Investigations have demonstrated that vitamin E succinate-induces concentration- and time-dependent inhibition of cancer cell growth via DNA synthesis blockage, induction of cellular differentiation, and induction of apoptosis (5, 6, 10, 13-15, unpublished data).

Inhibition of cell proliferation involves a GO/G1 cell cycle blockage, mediated, in part, by MAP kinases MEK1 and ERK1, and upregulation of the key cell cycle regulatory protein

p21(waf1/cip1) (30). Induction of differentiation is characterized by morphological changes, elevated beta casein message, expression of milk lipids, elevated cytokeratin 18 protein and down-regulation of Her2/neu protein (13). Differentiation is mediated, in part, by activation of MEK1, ERK1/2, and phosphorylation of the c-Jun protein (13, 14). Of the multiple apoptotic signaling events modulated by RRR-α-tocopherol succinate, especially noteworthy is its ability to convert Fas/Fas ligand non-responsive tumor cells to Fas/Fas ligand responsiveness and its ability to convert transforming growth factor-beta (TGF-α) non-responsive tumor cells to TGF-α responsiveness, with both restored pathways converging on JNK/c-Jun, followed by translocation of Bax protein to the mitochondria, induction of mitochondria permeability transition, followed by cytochrome c release to the cytoplasm, activation of caspases 9 and 3, cleavage of poly (ADP-ribose) polymerase (PARP), and apoptosis (15, 29, 31).

Vitamin E succinate is noteworthy not only for its induction of growth inhibitory effects on tumor cells but also for its lack of toxicity toward normal cells and tissues (2-7, 11). The use of a non-hydrolyzable vitamin E succinate derivative has shown that it is the intact compound and not either of its cleavage products (namely, RRR-α-tocopherol or succinic acid), that are responsible for the anti-proliferative effects (4). Thus, the anti-proliferative actions of this vitamin E derivative are considered to be due to non-antioxidant properties.

RRR-α-tocopheryl succinate (VES) is a derivative of RRR-α-tocopherol that has been structurally modified via an ester

linkage to contain a succinyl moiety instead of a hydroxyl moiety at the 6-position of the chroman head. This ester linked succinate moiety of RRR-α-tocopherol has been the most potent form of vitamin E affecting the biological actions of triggering apoptosis and inhibiting DNA synthesis. This form of vitamin E induces tumor cells to undergo apoptosis, while having no apoptotic inducing effects on normal cells. The succinated form of vitamin E is effective as an anticancer agent as an intact agent; however, cellular and tissue esterases that can cleave the succinate moiety, thereby converting the succinate form of RRR-α-tocopherol to the free RRR-α-tocopherol, render this compound ineffective as an anticancer agent. RRR-α-tocopherol exhibits neither antiproliferative nor proapoptotic biological activity in cells of epithelial or immune origin.

Construction of RRR-alpha-tocopherol or RRR-alpha-tocotrienol based compounds modified at the C6 position of the first ring of the chromonal head of alpha-tocopherol or alpha-tocotrienol via an ether linkage would provide compounds with potent anticancer properties. Cellular etherases have not been reported in cells; thus, such compounds will remain intact in cell culture as well as *in vivo*. In US 6,417,223 commercially available RRR-α-tocopherol in pure form was used as the starting material from which to synthesize vitamin E analogs. Modifications were made to three parts of the RRR-α-tocopherol molecule: to the number 6 carbon of the phenolic ring in the chroman head, to the chroman head, comprising the phenolic and heterocyclic rings, or to the phytyl tail. In RRR-α-tocopherol the number 6 carbon of

the phenolic ring has a hydroxyl (-OH) moiety which is critical for antioxidant activity..

Screening of the analogs for ability to induce apoptosis in a wide variety of human cancer cells, but not normal human cells, together with structure function analyses of the analogs suggest that tocopherol-based analogs of 29 Å in total length from H-bond donor to tip of phytyl tail, which itself is 17 Å in length, having a fully methylated closed phenolic ring, saturated closed heterocyclic ring, and a nonhydrolyzable acetic acid moiety attached to C6 of the phenolic ring with an ether linkage exhibit the most potent anti-cancer activity (Fig. 1).

The method of delivery of therapeutic agents, whether it is by oral, dietary, gavage, subcutaneous, intraperitoneal, topical, intravenous, intramuscular, respiratory, etc., has a major influence on levels and tissue distribution of drugs. U.S. Patent No. 6,090,407 demonstrates that anti-cancer drugs paclitaxel and camptothecin can be incorporated into liposomes for delivery to the respiratory tract of an individual via nebulization. The administration of these anti-cancer drugs by liposomal inhalation is a faster and more efficient means of delivery than either intramuscular injection or oral administration.

The use of a liposomal aerosol for delivery of the plant alkaloid 9-nitrocamptothecin is a superior method for inhibition of human breast (28), colon, and lung cancer xenografts in immune compromised nude mice when compared to delivery of 9-nitrocamptothecin by intramuscular injection. Levels of 9-nitrocamptothecin, after thirty minutes by aerosol liposome method of delivery, in the lungs, liver and brain were 310 ng/g,

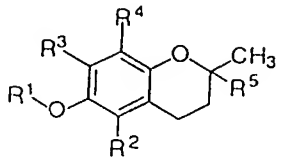
192 ng/g, and 61 ng/g, respectively; whereas, levels of 9-nitrocamptothecin, after thirty minutes by intramuscular delivery, in the lungs, liver, and brain were 2-4 ng/g, 136 ng/g, and 0, respectively (16). In addition, this method of delivery appears to be highly effective against pulmonary metastasis of melanoma and osteosarcoma in mice (18). Of major importance, aerosol delivery of drugs shows increased efficacy and is well tolerated by humans (19). Thus, the aerosol liposome method for delivery of drugs is effective in achieving higher levels and greater tissue distribution.

Additionally, the inventors have recognized a need for further effective methods of liposomal delivery of vitamin E based anticancer drugs that provide for longer retention, higher drug concentration, reduced systemic toxicity and reduced dosage requirements. Thus, the prior art is deficient in the lack of an effective means of delivering vitamin E based anticancer drugs via liposomesal to an individual. Specifically, the aerosol liposomal delivery or administration by gavage of liposomal compositions of vitamin E based anticancer drugs is desired. The present invention fulfills this long-standing need and desire in the art.

SUMMARY OF THE INVENTION

In one embodiment of the present invention, there is provided a method for treating a cell proliferative disease comprising the step of delivering a composition comprising a vitamin E based anti-cancer compound contained within a

delivery vesicle to an individual in need of such treatment where the compound has a structural formula



where R^1 is a hydrogen or a carboxylic acid; R^2 and R^3 are hydrogen or R^4; R^4 is methyl; and R^5 is alkyl or alkenyl.

In another embodiment of the present invention, there is provided a vesicle for delivery of the vitamin E based anticancer compound as described herein.

Other and further aspects, features, benefits, and advantages of the present invention will be apparent from the following description of the presently preferred embodiments of the invention given for the purpose of disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

So that the matter in which the above-recited features, advantages and objects of the invention, as well as others which will become clear, are attained and can be understood in detail, more particular descriptions of the invention are briefly summarized. above may be had by reference to certain

embodiments thereof which are illustrated in the appended drawings. These drawings form a part of the specification. It is to be noted; however, that the appended drawings illustrate preferred embodiments of the invention and therefore are not to be considered limiting in their scope.

Figure 1 is a model of R,R,R-α-tocopherol based analogs depicting the structural elements required for potent anti-cancer activity. The R^1 group side chain at C6 must be of a length such that the total length of the molecule does not exceed 29Å.

Figure 2 compares the anticancer effects of natural alpha- and gamma-tocopherols, natural alpha-tocotrienol, tocotrienol enriched fraction (TRF), synthetic tocopherols and synthetic tocopherol derivatives on 66 cl.4 GFP mammary tumor cells.

Figures 3A-3D depict α-TEA induced apoptosis. **Figure 3A:** 66 cl.4-GFP murine mammary cells were treated with 10 μg/ml of α-TEA or vitamin E succinate (positive control) or untreated and cultured for 3 days. Cells were harvested, nuclei were labeled with the fluorescent DNA-binding dye DAPI and cells were examined using a Zeiss ICM 405 fluorescent microscope (x400), using a 487701 filter. Nuclei of cells with condensed chromatin or fragmented nuclei were scored as apoptotic. Data are representative of numerous experiments. **Figures 3B/3C:** Analyses of nuclei of DAPI stained cells show α-TEA to induce apoptosis in a concentration- and time- dependent manner. Data are depicted as mean ± S.D. of three experiments. **Figure 3D:** Additional evidence of α-TEA induction of apoptosis by poly (ADP-ribose) polymerase (PARP) cleavage. 66 cl.4-GFP cells were

treated with 5, 10, or 20 μg/ml α-TEA for 48 hours, cellular lysates were analyzed for PARP cleavage product (p84) by western immunoblot analyses. Data are representative of 3 separate experiments.

Figures 4A and **4B** depict that α-TEA induces 66 cl.4 cells to undergo apoptosis *in vivo*. α-TEA induction of apoptosis was determined using 5 μm tumor sections derived from liposomal α-TEA/aerosol treatment and liposome aerosol control group animals (N = 4). Apoptotic cells were determined using ApopTag *In Situ* Apoptosis Detection kit (Intergen, Purchase, NY). **Figure 4A** compares the number of apoptotic nuclei in liposomal α-TEA aerosol and aerosol treated controls. **Figure 4B** depicts positive stained apoptotic cells in tumor sections from liposomal α-TEA aerosol and control treated mice.

Figure 5 depicts α-TEA inhibition of 66 cl.4-GFP clonal growth. Treatment of 66 cl.4-GFP cells (plated at 600 cells/tissue culture plate) with α-TEA at 1.25, 2.5, and 5 μg/ml for 10 days inhibited colony formation. Cells were stained with methylene blue and the number of colonies in treatment and control groups were counted.

Figure 6 depicts body weight of balb/c mice implanted with 66 cl.4 GFP murine mammary tumor cells and treated with liposome/α-TEA composition delivered via aerosol. Animal weight is monitored from day nine after implantation.

Figure 7 depicts serum and tissue levels of α-TEA in balb/c mice 0, 2, 6, or 24 hours after liposomal/α-TEA **1** aerosol treatment ended.

Figure 8 depicts tumor weights in balb/c mice implanted with 66 cl.4 GFP murine mammary tumor cells and treated with liposomal/α-TEA delivered via aerosol. Tumor weight is monitored from day nine after implantation.

Figures 9A and **9B** depict the inhibition of tumor growth and micrometatases with liposomal aerosol α-TEA. **Figure 9A:** 66 cl.4-GFP cells at 2 x 10^5/mouse were injected into the inguinal area at a point equal distance between the 4th and 5th nipples. Nine days after tumor inoculation, mice (10/group) were not treated or treated daily with liposomal α-TEA/aerosol or aerosol only for 17 days. Tumor volume/mouse was determined at two-day intervals. Tumor volume (mm^3) are depicted as mean ± S. E. **Figure 9B:** At necropsy, the number of fluorescent micrometastases in the left lung lobe from liposomal α-TEA/aerosol (8 mice), aerosol only (10 mice), and untreated mice (10 mice) were determined using a Nikon fluorescent microscope (TE-200; 200X), and Image Pro-Plus software was used for determining size of micrometastases. Data are depicted as mean ± S. E.

Figure 10 depicts the inhibition of tumor growth of 66 cl.4 GFP mammary tumor cells by liposomal α-TEA and liposomal vitamin E succinate (VES) delivered via aerosol nebulization.

Figure 11 depicts tumor weights in balb/c mice implanted with 66 cl.4 GFP murine mammary tumor cells and treated with liposomal/ α-TEA delivered via gavage. Tumor weight is monitored from day nine after implantation. Figure 11 differs from Figure 8 only in that the mice were treated daily by

gavage with 5 mg α-TEA in peanut oil or received peanut oil only, and were treated for only 13 days.

Figures 12A and **12B** depict that α-TEA administered by gavage does not inhibit tumor growth at the site of inoculation but does inhibit lung micrometastases. Tumor volume and micrometastases data determinations were as described in the legend to Figures 9A and 9B.

Figures 13 depicts the inhibition of tumor growth of 66 cl.4 GFP mammary tumor cells by liposomal α-TEA and liposomal vitamin E succinate (VES) delivered via gavage.

Figures 14A and **14B** depict the inhibition of lung metastases (Figure 14A) and lymphnode metastases (Figure 14B) of 66 cl.4 GFP mammary tumor cells by liposomal α-TEA and liposomal vitamin E succinate (VES) delivered via gavage.

Figure 15 depicts the inhibition of tumor growth of MDA-MB-435 breast cancer cells by liposomal α-TEA delivered via gavage.

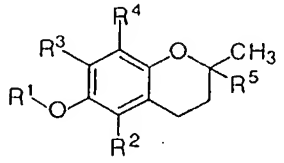
Figures 16A-16C depict the apoptotic effects on **of** α-TEA or α-TEA/cisplatin in combination on the A2780 **cisplatin**-sensitive cell line and on cisplatin-insensitive cp-70 human ovarian cancer cells. **Figure 16A**: Apoptotic effect *in vitro* of α-TEA on the A2780 and cp-70 human ovarian cancer cell lines. **Figure 16B**: α-TEA restores cisplatin sensitivity to cp-70 *in vitro*. **Figure 16C**: the growth inhibitory effect of both α-TEA and cisplatin in combination on A2780 and cp-70 *in vivo*. In vivo data show that α-TEA converts cp-70 cisplatin cells to cisplatin

sensitivity, and that combinations of α-TEA + cisplatin reduce the growth of cp-70 ovarian cell xenografts in nude mice.

Figure 17 depicts the effect of α-TEA, methylselenocysteine and trans-resveratrol on MDA-MB-435 GFP FL breast cancer tumor growth *in vivo*.

DETAILED DESCRIPTION OF THE INVENTION

In one embodiment of the present invention, there is provided a method for treating a cell proliferative disease comprising the step of delivering a composition comprising a vitamin E based anti-cancer compound contained within a delivery vesicle to an individual in need of such treatment where the compound has a structural formula



where R^1 is a hydrogen or a carboxylic acid; R^2 and R^3 are hydrogen or R^4; R^4 is methyl; and R^5 is alkyl or alkenyl. In all aspects of this embodiment the vitamin E based anticancer compounds may be a tocopherol such as β- tocopherol, γ-tocopherol, δ-tocopherol, or 2,5,7,8-tetramethyl-(2R-(4R,8R,12-trimethyltridecyl) chroman-6-yloxy) acetic acid. Additionally, the vitamin E based anticancer compounds may be tocotrienol such as

α- tocotrienol, β- tocotrienol, γ- tocotrienol, δ- tocotrienol, or tocotrienol enriched fraction, or a synthetic vitamin E based compound such as dl-α-tocopherol, dl-α-tocopherol acetate, dl-α-tocopherol nicotinate, or dl-α-tocopherol phosphate.

In this embodiment the delivery vesicle may be a liposome comprising a lipid, a nanoparticle, a microsphere or a niosome. A representative example of a suitable lipid in the liposome is 1,2-dilauroyl-sn-glycero-3-phosphocholine. A preferred example is a liposome with a final concentration of the vitamin E based anti-cancer compound in the liposome that is no greater than 20.0 mg/ml. The vitamin E based compound/delivery vesicle may be delivered via aerosol nebulization, an aerosol inhaler, gavage, oral ingestion, orally by soft gel capsule, a transdermal patch, subcutaneous injection, intravenous injection, intramuscular injection, or intraperitoneal injection. A preferred means of delivery is a liposomal aerosol via a jet nebulizer.

In an aspect to this embodiment the method may further comprise the step of administering a second composition of an anticancer drug contained within a delivery vehicle. The second composition may be administered in combination with or sequentially to the vitamin E based compound/delivery vesicle composition. When administration of the compositions is combined, the vitamin E based compound and the anticancer drug may be contained within the same delivery vesicle. Representative examples of anticancer drugs are anticancer drug is 9-nitrocamptothecin, cisplaten, paclitaxel, doxirubicin, or celecoxib.

The vitamin E based anti-cancer compounds of the instant invention exhibit an anti-proliferative effect comprising apoptosis, DNA synthesis arrest, cell cycle arrest, or cellular differentiation. In this embodiment the quantitative and/or qualitative analysis of the antiproliferative effect may be determined by detecting a biomarker. A preferred example of a biomarker is the cell proliferation marker KI-67. Alternatively, a immunohistochemical assay may be used.

Delivery of the vitamin E based or other anti-cancer compounds of the present invention may be used to treat neoplastic diseases and non-neoplastic diseases. Representative examples of neoplastic diseases are ovarian cancer, cervical cancer, endometrial cancer, bladder cancer, lung cancer, cervical cancer, breast cancer, prostate cancer, testicular cancer, gliomas, fibrosarcomas, retinoblastomas, melanomas, soft tissue sarcomas, osteosarcomas, colon cancer, carcinoma of the kidney, pancreatic cancer, basal cell carcinoma, and squamous cell carcinoma. Representative examples of non-neoplastic diseases are selected from the group consisting of psoriasis, benign proliferative skin diseases, ichthyosis, papilloma, restinosis, scleroderma and hemangioma, and leukoplakia.

Methods of the present invention may be used to treat non-neoplastic diseases that develop due to failure of selected cells to undergo normal programmed cell death or apoptosis. Representative examples of diseases and disorders that occur due to the failure of cells to die are autoimmune diseases. Autoimmune diseases are characterized by immune cell destruction of self cells, tissues and organs. A representative

group of autoimmune diseases includes autoimmune thyroiditis, multiple sclerosis, myasthenia gravis, systemic lupus erythematosus, dermatitis herpetiformis, celiac disease, and rheumatoid arthritis. This invention is not limited to autoimmunity, but includes all disorders having an immune component, such as the inflammatory process involved in cardiovascular plaque formation, or ultra violet radiation induced skin damage.

Methods of the present invention may be used to treat disorders and diseases that develop due to viral infections. Representative examples of diseases and disorders that occur due to viral infections are human immunodeficiency viruses (HIV). Since vitamin E based compounds work on intracellular apoptotic signaling networks, a delivery vesicle, such as a liposomal aerosol, containing the vitamin E based anti-cancer compounds of the present invention have the capacity to impact signal transduction of any type of external cellular signal such as cytokines, viruses, bacteria, toxins, heavy metals, etc.

In another embodiment of the present invention, there is provided a vesicle for delivery of a vitamin E based anticancer compound contained therein. In an aspect of this embodiment the vesicle may further comprise an anticancer drug. In a preferred aspect, the delivery vesicle is a liposome having a ratio of vitamin E based anticancer compound to lipid is about 1:3 wt:wt. The vitamin E based anti-cancer compounds, the anticancer drugs, the types of vesicles and the delivery methods may be those as disclosed *supra*.

The following definitions are given for the purpose of facilitating understanding of the inventions disclosed herein. Any terms not specifically defined should be interpreted according to the common meaning of the term in the art.

As used herein, the terms "aerosol" "gavage", "liposome" "delivery vesicle" and "vesicle" shall include different chemical compositions for vesicle/liposome preparations and different methodologies for aerosol dispersal or oral delivery of these preparations.

As used herein, the term "individual" shall refer to animals and humans.

As used herein, the term "biologically inhibiting" or "inhibition" of the growth of syngenic tumor grafts shall include partial or total growth inhibition and also is meant to include decreases in the rate of proliferation or growth of the tumor cells. The biologically inhibitory dose of the composition of the present invention may be determined by assessing the effects of the test element on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell culture or any other method known to those of ordinary skill in the art.

As used herein, the term "inhibition of metastases" shall include partial or total inhibition of tumor cell migration from the primary site to other organs, specifically the lungs in the data provided above. The biological metastatic inhibitory dose of the composition of the present invention may be determined by assessing the effects of the test element on target malignant or abnormally proliferating cell growth in tissue culture, tumor

growth in animals and cell culture or any other method known to those of ordinary skill in the art.

As used herein, the term "inhibition of angiogenesis" shall include partial or total inhibition of tumor blood vessel formation or reduction in blood carrying capacity of blood vessels supplying blood to tumors.

As used herein, the term "induction of programmed cell death or apoptosis" shall include partial or total cell death with cells exhibiting established morphological and biochemical apoptotic characteristics. The dose of the composition of the present invention that induces apoptosis may be determined by assessing the effects of the test element on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell culture or any other method known to those of ordinary skill in the art.

As used herein, the term "induction of DNA synthesis arrest" shall include growth arrest due to treated cells being blocked in GO/G1, S, or G2/M cell cycle phases. The dose of the composition of the present invention that induces DNA synthesis arrest may be determined by assessing the effects of the test element on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell culture or any other method known to those of ordinary skill in the art.

As used herein, the term "induction of cellular differentiation" shall include growth arrest due to treated cells being induced to undergo cellular differentiation as defined by established morphological and biochemical differentiation characterization, a stage in which cellular proliferation does not

occur. The dose of the composition of the present invention that induces cellular differentiation may be determined by assessing the effects of the test element on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell culture or any other method known to those of ordinary skill in the art.

As used herein, "α-TEA" shall include an RRR-α-tocopherol ether-linked acetic acid analog which is a non-hydrolyzable ether analog of RRR-α-tocopherol, i.e., 2,5,7,8-tetramethyl-2R-(4R,8R-12-trimethyltridecyl)chroman-6-yloxy acetic acid which can also be abbreviated as RRR-α-tocopheryloxyacetic acid.

Provided herein are methods of treating cell proliferative diseases via aerosol delivery of a liposomal composition or via delivery by gavage of a liposomal composition comprising a natural or synthetic vitamin E based anti-cancer drug and a lipid. The vitamin E based compounds of the instant invention exhibit an anti-proliferative effect; representative examples of these anti-proliferative effects are apoptosis, DNA synthesis arrest, cell cycle arrest, or cellular differentiation. These compounds exhibit potent anti-metastatic effects and do not exhibit toxicity to normal cells and tissue *in vivo* when administered by a clinically relevant route such as aerosol delivery or gavage.

Optionally, the vitamin E based anticancer drug and a second anti-cancer drug, such as, but not limited to, 9-nitrocamptothecin (9NC), doxorubicin, paxitaxol, celecoxib, or cisplatin, in a liposomal composition or administered via another

route, such as intraperitoneal injection, can be administered in combination or sequentially with α-TEA. For example, 9-nitrocamptothecin, as a topoisomerase-1 inhibitor which causes DNA single-strand breaks that are converted into DNA double strand breaks during DNA replication, has a different cell killing mechanism from α-TEA. Furthermore, 9NC can activate a traditional CD95/CD95Ligand (FADD/caspase 8) dependent apoptotic pathway (29) which might synergize with the nontraditional pathway, i.e., CD95/Daxx/JNK/Mitochondria. This provides for enhanced cell killing.

These vitamin E based compounds of the instant invention include natural or synthetic tocopherols or tocotrienols and derivatives thereof having chemical funtionalization at position R^1 of the chroman structure and position R^5 of the phytyl or isoprenyl side chains. Preferably, R^1 is a carboxylic acid, e.g. acetic acid. Generally, synthesis of these compounds is accomplished by reacting R,R,R-alpha-tocopherol with the appropriate bromoalkanoic acid using methods standard in the art.

Derivatives of the natural tocopherols and tocotrienols are non-hydrolizable by cellular esterases and, although not limited to such a C6 side chain, preferably have an acetic acid moiety in this position. A preferred compound that fulfills the structural elements required for potent anti-cancer activity is α-TEA which differs from RRR-α-tocopherol by an acetic acid moiety linked to the phenolic oxygen at carbon 6 of the chroman head by an ether linkage. VES differs from α-TEA in that a succinic acid moiety is linked by an ester linkage to the phenol at carbon 6 of the chroman head. Since the antioxidant properties of the parent

compound, RRR-α-tocopherol, reside in the –OH moiety at carbon 6, the anti-tumor properties of α-TEA are not antioxidant mediated.

The present invention may be used as a therapeutic agent. The methods of the present invention may be used to treat any animal. Most preferably, the methods of the present invention are useful in humans. Generally, to achieve pharmacologically efficacious cell killing and anti-proliferative effects, the liposomal/anti-cancer compound compositions may be administered in any therapeutically effective dose. The dosage administered is dependent upon the age, clinical stage and extent of the disease or genetic predisposition of the individual, location, weight, kind of concurrent treatment, if any, and nature of the pathological or malignant condition. Preferably, the dosage is from about 0.1 mg/kg to about 100 mg/kg. A person having ordinary skill in this art would readily be able to determine, without undue experimentation, the appropriate dosages.

In vivo studies of tumor growth and metastasis of human tumor cells either ectopically or orthotopically transplanted into immune compromised animals, such as nude mice, or *in vivo* studies employing well recognized animal models provides pre-clinical data for clinical trials. Without being limited to these animal models, such *in vivo* studies can focus on other neoplastic and non-neoplastic models of cell proliferative diseases. For example, the metastatic non-estrogen responsive MDA-MB-435 breast cancer cells, 66 cl.4 GFP cells or cisplatin resistant cp-70 human ovarian cancer cells can be used.

The use of additional novel tocopherol, tocotrienol, and other chroman derivatives with or without derivatives of

saturated phytyl or unsaturated isoprenyl side chains or analogs thereof in a liposomal composition for delivery to an individual such as aerosol delivery to the respiratory tract or delivery via gavage, is specifically contemplated. These molecules include chemical funtionalization of positions R^1–R^5 of the chroman structure, and chemical functionalization of the phytyl and isoprenyl side chains, particularly compounds based on tocopherols and tocotrienols. Additionally, compounds with heteroatom substitutions (N or S) for the chroman ring oxygen and the oxygen of the 6-hydroxy group are contemplated.

Using alkylation chemistry, a large number of compounds containing different R^1 groups can be synthesized, particularly when X is oxygen. After alkylation, further chemical modification of the R^1 groups permits the synthesis of a wide range of novel compounds. R^1 substituents can be alkyl, alkenyl, alkynyl, aryl, heteroaryl, carboxylic acid, carboxylate, carboxamide, ester, thioamide, thiolacid, thiolester, saccharide, alkoxy-linked saccharide, amine, sulfonate, sulfate, phosphate, alcohol, ethers and nitriles.

Bromination of the benzylic methyl groups of the chroman group provide intermediates that permit variation of the R^2, R^3 and R^4 groups. Substituents for R^2 and R^3 may be hydrogen or additional substituents for R^4, e.g., methyl, benzyl carboxylic acid, benzyl carboxylate, benzyl carboxamide, benzylester, saccharide and amine. Variation of group R^5, such as alkyl, alkenyl, alkynyl, aryl, heteroaryl, carboxyl, amide and ester, is also possible, particularly when starting from the commercially

available 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

When a heteroatom substitution of nitrogen for the chroman ring oxygen occurs, than nitrogen may be substituted with R^6 which is hydrogen or methyl. Variation of X to groups other than oxygen, which is the identity of X in tocopherols and tocotrienols, can be accomplished using palladium chemistry (for X = CH_2) and nucleophilic aromatic substitution (for X = N or S). Other possible modifications to the chroman structure include unsaturation at the 3-4 positions and ring contraction to produce a five-membered furanyl ring.

It is also contemplated that other lipids may be used in the liposomal composition. Any lipid that can incorporate these vitamin E based anti-cancer compounds or other anticancer compounds and deliver a therapeutic dosage would be suitable. Different methods of liposomal delivery are used such as via aerosolization or gavage can be used. For example, methods of aerosolization and nebulization for liposomal delivery or methods of liposomal administration by gavage can be used.

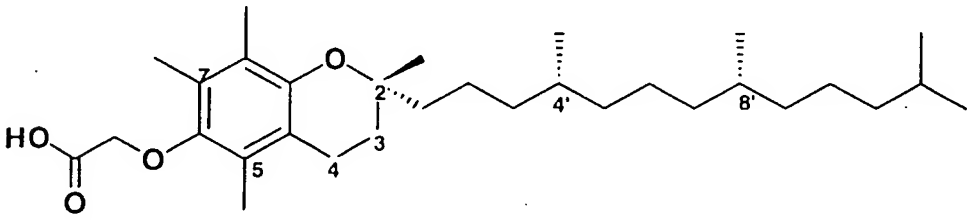
It is also contemplated that α-TEA or other derivatized tocopherols or tocotrienols may be used in preparations of niosomes, microspheres or nanoparticles and delivered as a therapeutic dose via aerosol inhalation or aerosol nebulization to the respiratory tract or administered by gavage, topical application, subcutaneous, intraperitoneal, intravenous, intramuscular or other established methods for administration. For example, but not limited to, a liposomal or nanoparticle formulation of α-TEA in a soft gel capsule for oral delivery may be

ideally suited for in human chemoprevention. Nanoparticle formulations administered orally as soft gel capsules might provide longer retention in the digestive tract and perhaps greater uptake. Furthermore, nanoparticle formulations may be useful for delivery by inhalation. Liposomal or nanoparticle preparations may be useful in a transdermal delivery system such as in a patch.

The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion.

EXAMPLE 1

Synthesis and characterization of 2,5,7,8-tetramethyl-(2R-(4R,8R,12-trimethyltridecyl) chroman-6-yloxy) acetic acid (α-TEA)



For scaled-up production, α-TEA was prepared as follows. NaH (5.0 g, 124.9 mmol) was suspended in dry THF (300 ml) and stirred under argon at 0 °C for 10 min. prior to the addition via cabbula of α-tocopherol (41.3 g, 96.1 mmol) that was dissolved in 100ml of dry THF. This mixture was stirred at 0 °C for 15 min while under argon pressure, then ethyl bromoacetate

(19.26 g, 115.3 mmol) was added via syringe. The reaction was monitored by TLC (hexane : ethyl acetate = 10 : 1 , R_f = 0.65) and was completed in 3.5 hours. The reaction mixture was diluted with 150 ml of CH_2Cl_2, washed with saturated NaCl solution (150 ml x 3) until the organic phase was clear, dried over anhydrous Na_2SO_4, and the solvent removed under a reduced pressure. The crude product still contained a small amount of free α-tocopehrol which could be removed by column chromatography on silica gel using hexane: ethyl acetate (30:1 to 20:1) to yield pure product α-TEA ethyl ester (41.6 g, 84%).

The α-TEA ethyl ester (21.0 g, 40.7 mmol) was dissolved in 250 ml of THF, then 75 ml of 10% KOH (122.1 mmol) was added and the mixture stirred at room temperature for 6 hours. The reaction was monitored by TLC ($CHCl_3$: MeOH : CH_3COOH = 97 : 2.5 : 0.5, R_f = 0.18) and was quenched with 100 ml of water. The solution was adjusted to pH 3 using 1N HCl and the product extracted with CH_2Cl_2 (100 ml x 4), washed with saturated NaCl solution, dried over Na_2SO_4 and the solvent was removed under a reduced pressure, providing the final product α-TEA as a white waxy solid (18.5 g, 93%). Melting point: 54-55°C; Molecular weight: 488.8

EXAMPLE 2

Murine Mammary Tumor Cell Line

66 cl.4-GFP cells are a mouse mammary tumor cell line originally derived from a spontaneous mammary tumor in a Balb/cfC3H mouse and later isolated as a 6-thioguanine-resistant

clone (20, 21). Subsequently these cells were stably transfected with the enhanced green fluorescent protein (GFP). 66 cl.4-GFP cells are highly metastatic with 100% micrometastases to the lungs. Prior to use in these studies, cells were sent to the University of Missouri Research Animal Diagnostic and Investigative Laboratory (RADIL; Columbia, MO) where they were certified to be pathogen free.

66 cl.4 GFP cells were maintained as monolayer cultures in growth media: McCoy's media (Invitrogen Life Taechnologies, Carlsbad, CA.) supplemented with 10% fetal bovine serum (FBS, Hyclone Lab, Logan, UT), 100 µg/ml streptomycin, 100 IU/ml penicillin, 1 X (vol/vol) non-essential amino acids, 1X (vol/vol) MEM vitamins, 1.5 mM sodium pyruvate, and 50 µg/ml gentamycin (Sigma Chemical Co., St. Louis, MO). Treatments were given using this same McCoy's supplemented media expect FBS content was reduced to 5%. Cultures were routinely examined to verify absence of mycoplasma contamination.

EXAMPLE 3

Determination of apoptosis by morphological evaluation of DAPI-stained nuclei

Apoptosis was determined using previously published procedures (22). Briefly, 1 x10^5 cells/well in 12-well plates were cultured overnight to permit attachment. Next, the cells were treated with α-TEA, vitamin E succinate (Sigma) or ethanol control (0.1% ethanol F.C. vol/vol) in experimental media at various

concentrations of α-TEA and vitamin E succinate for various time intervals. After treatment, floating cells plus scraped-released adherent cells were pelleted by centrifugation for 5 min at 350 X *g*, washed one time with phosphate buffered saline (PBS; 137 mM NaCl, 2.7 mM KCL, 10.4 mM Na_2HPO_4, 10.5 mM KH_2PO_4; pH 7.2), and stained with 2 μg/ml of 4',6-diamidino-2-phenylindole dihydrochloride (DAPI, Boehringer Mannheim, Indianapolis, IN) in 100% methanol for 15 min at 37°C.

Cells were viewed at 400X magnification with a Zeiss ICM 405 fluorescent microscope using a 487701 filter. Cells in which the nucleus contained condensed chromatin or cells exhibiting fragmented nuclei were scored as apoptotic. Data are reported as percentage of apoptotic cells per cell population, i.e., number apoptotic cells/total number of cells counted. Three different microscopic fields were examined and 200 cells counted at each location for a minimum of 600 cells counted per slide. Apoptotic data are presented as mean ± S.D. for three independently conducted experiments.

EXAMPLE 4

Western Immunoblot Detection of Poly (ADP-Ribose) Polymerase (PARP) Cleavage Fragment

Poly (ADP-Ribose) Polymerase cleavage was analyzed as an alternate method for detecting apoptosis. 66 cl.4-GFP cells were treated as described above for the DAPI assay. Following the PBS wash, cells were suspended in lysis buffer (1X PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl

sulfate (SDS), 1 μg/ml leupeptin, 1 μg/ml aprotinin, 1mM dithiothreitol (DTT), 2 mM sodium orthovanadate, 10 μg/ml phenylmethylsulfonyl fluoride (PMSF)) for 30 min at 4°C, vortexed, and supernatants collected by centrifugation at 15,000 X *g* for 20 min. Protein concentrations were determined using the Bio-Rad (Bradford) protein assay (Bio-Rad Laboratories, Hercules, CA), and samples (100 μg/lane) resolved on 7.5% SDS-polyacrylamide gels electrophoresed under reducing conditions.

Proteins were electroblotted onto a nitrocellulose membrane (0.2 μM pore Optitran BA-S-supported nitrocellulose; Schleicher and Schuell, Keene, NH). After transfer, membranes were blocked with blocking buffer [25 mM Tris-HCl (pH 8.0), 125 mM NaCl, 0.5% Tween-20, and 5% non-fat dry milk] for 45 min at room temperature. Immunoblotting was performed using 1 μg of primary rabbit anti-human Poly (ADP-Ribose) Polymerase antibody [PARP (H-250), Santa Cruz Biotechnology, Santa Cruz, CA], and horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin was used as the secondary antibody (Jackson Immunoresearch Laboratory, West Grove, PA) at a 1:3,000 dilution. Horseradish peroxidase-labeled bands from washed blots were detected by enhanced chemiluminescence (Pierce, Rockford, IL) and autoradiography (Kodak BioMax film; Rochester, NY).

EXAMPLE 5

Colony-forming Assay

66 cl.4-GFP cells were seeded at 600 cells per 35 X 10 mm tissue culture plate (Nunclon, Rochester, NY) and allowed to

adhere over night at 37° C. The next day, growth media were removed and replaced with treatment media containing α-TEA at 1.25, 2.5, 5, and 10 μg/ml, ethanol control (0.1% ethanol F.C. vol/vol), or media alone (untreated). Treatments were left on the cells for 10 days without change of media. After 10 days, media were removed and plates were washed with PBS three times. Cells were stained for 30 min with 1% methylene blue in PBS and colonies > 0.5 mm manually counted.

EXAMPLE 6

Balb/c Mice

Female Balb/cJ mice at 6 weeks of age (25 gm body weight) were purchased from Jackson Labs (Bar Harbor, ME), and were allowed to acclimate at least one week. Animals were housed at the Animal Resource Center at the University of Texas at Austin at 74 ± 2°F with 30-70% humidity and a 12 h alternating light-dark cycle. Animals were housed 5/cage and given water and standard lab chow *ad libitum*. Guidelines for the humane treatment of animals were followed as approved by the University of Texas Institutional Animal Care and Use Committee.

EXAMPLE 7

Tumor Cell Inoculation

66 cl.4-GFP cells were harvested by trypsinization, centrifuged, resuspended in McCoy's media, containing no supplements at a density of 2 X 10^5/100μl. Mice were injected in

the inguinal area at a point equal distance between the 4th and 5th nipples on the right side using a 23 gauge needle.

50 mice were assigned (10 per group) to aerosol treatment, aerosol control, oral treatment, oral control, or to the no treatment group so that the average tumor volume for all groups were closely matched. Each group had a range of 2 x 2-4 x 4 mm tumors at the start of treatments which were begun nine days following tumor cell inoculation. Ten additional mice, not injected with tumor cells, were treated with aerosol or oral α-TEA (5 each) for 17 days, removed from treatment and observed for an additional 11 months to evaluate long term safety. Tumors were measured using calipers every other day, and volumes were calculated using the formula: volume (mm^3) = [width $(mm)^2$ X length (mm)] /2. Body weights were determined weekly.

EXAMPLE 8

Preparation and Administration of α-TEA Solubilized in Peanut Oil for Delivery by Gavage

α-TEA was dissolved in 100% ethanol (400 mg/ml) and then mixed with peanut oil (100% peanut oil; nSpired Natural Foods, San Leandro, CA) at a ratio of 1:8 (v/v). Control treatment consisted of equivalent amounts of ethanol and peanut oil as contained in the α-TEA treatment. The mixtures were vortexed vigorously then stored at 4° C until used. α-TEA /peanut oil mixture was brought to room temperature and administered by gavage 100 µl/mouse per day. This corresponds to a final concentration of 5 mg α-TEA/mouse/day.

EXAMPLE 9

Preparation of liposomal composition containing 2,5,7,8-tetramethyl-(2R-(4R,8R,12-trimethyltridecyl) chroman-6-yloxy) acetic acid (α-TEA)

An α-TEA/liposome ratio of 1:3 (w/w) was determined empirically to be optimal by methods previously described (17). To prepare the α-TEA/lipid combination, the components were first brought to room temperature. The lipid [1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC); Avanti Polar-Lipids, Inc., Alabaster, AL] at a concentration of 120 mg/ml was dissolved in tertiary-butanol (Fisher Scientific, Houston, TX) then sonicated to obtain a clear solution. α-TEA at 40 mg/ml was also dissolved in tertiary-butanol and vortexed until all solids were dissolved. The two solutions were then combined in equal amounts (v:v) to achieve the desired ratio of 1:3 α-TEA/liposome, mixed by vortexing, frozen at –80° C for 1-2 h, and lyophilized overnight to a dry powder prior to storing at –20°C until needed. Each treatment vial contained 75 mg α-TEA.

EXAMPLE 10

Aerosolization and administration of liposome containing 2,5,7,8-tetramethyl-(2R-(4R,8R,12-trimethyltridecyl) chroman-6-yloxy) acetic acid (α-TEA)

Aerosol was administered to mice as previously described (17). Briefly, an air compressor (Easy Air 15 Air

Compressor; (Precision Medical, Northampton, PA) producing a 10L/min airflow was used with an AeroTech II nebulizer (CIS-US, Inc. Bedford, MA) to generate aerosol. The particle size of α-TEA liposome aerosol discharged from the AeroTech II nebulizer was determined by the Anderson Cascade Impactor to be 2.01 μm mass median aerodynamic diameter (NMAD), with a geometric standard deviation of 2.04. About 30% of such particles when inhaled will deposit in the respiratory tract of the mouse and the remaining 70% will be exhaled (17).

Prior to nebulization, the α-TEA/lipid powder was brought to room temperature then reconstituted by adding 3.75 ml distilled water to achieve the final desired concentration of 20 mg/ml α-TEA. The mixture was allowed to swell at room temperature for 30 min with periodic inversion and vortexing, and then added to the nebulizer. This α-TEA formulation can be administered orally by gavage at levels of 4 mg α-TEA/0.1ml. Mice were placed in plastic cages (7 x 11 x 5 in.) with a sealed top in a safety hood. Aerosol entered the cage via a 1 cm accordion tube at one end and discharged at the opposite end, using a one-way pressure release valve. Animals were exposed to aerosol until all α-TEA/liposome was aerosolized, approximately 15 min.

EXAMPLE 11

Aerosol Characteristics of α-TEA Incorporated into Liposomes

HPLC analyses were conducted on α-TEA liposomes recovered from aerosol in the All Glass Impinger (Ace Glass Co.,

Vineland, NJ). The delivered dosage = concentration (μg/L) x mouse minute volume (1-min/kg) x duration of delivery (min) x estimated deposited fraction (30%; 17). Based on this formula, we estimate that approximately 36 μg g of α-TEA was deposited in the respiratory tract of each mouse each day (316.2 μg g/L x 1 min/kg x 15 min x 0.30 = 1,422.9 μg g/kg/day.

EXAMPLE 12

HPLC Analyses of α-TEA in Tumors

Tumors were removed during necropsy and half of each tumor was quick frozen in liquid nitrogen then stored at –70°C until HPLC analyses were performed. Tumors were processed for HPLC analyses by homogenization and hexane extraction of lipids. Briefly, weighed tumors were placed in 5 ml disposable conical-bottom tubes (Sarstedt, Newton, NC) along with 5-7 Kimble solid glass beads (4mm), 1 ml 1% SDS in water, 1-2 ml 100% ethanol and 1 ml hexane. Samples were then mixed using a Crescent Wig-L-Bug (model 3110B Densply International, Elgin, IL) twice for 1 min each. Samples were then centrifuged for 5 min at 1,000 rpm, and the organic layer collected, the aqueous layer was mixed with hexane again, vortexed, centrifuged, and processed twice more before the organic layer was dried down under nitrogen. Samples were run immediately. Reverse phase HPLC analyses with fluorometric detection of the tocopherol ether analog were conducted as described by Tirmenstein, M.A., et al. (23).

EXAMPLE 13

Lung and lymph node Metastastes

Metastastic lesions in the five lung lobes were counted visually at time of sacrifice. Fluorescent green micro-metastatic cancer cell colonies in the left lung lobe and lymph nodes were counted using a Nikon fluorescence microscope (TE-200) with a 20 x objective (200 x magnification) and Image-Pro Plus (version 4.1; Media Cybernetics, Silver Spring, MD) software associated with the microscope. Fluorescent lesions were separated into three size grouping: <5 μm , 5-10 μm and >10 μm.

EXAMPLE 14

TUNEL Assay for Detection of Apoptosis in Vivo

Deparaffinized sections (5 μm) of tumor tissue were used to assess apoptosis using reagents supplied in the ApopTag *In Situ* Apoptosis Detection kit (Intergen, Purchase, NY) according to the manufacturer's instructions. Nuclei that stained brown were scored as positive for apoptosis and those that stained blue were scored as negative. At least sixteen 400X microscopic fields were scored per tumor. Data are presented as the mean ± S. E. number of apoptotic cells counted in three separate tumors from each group. Pictures of tumor tissue were taken at 1000X magnification.

EXAMPLE 15

H&E Staining of Tumor Tissue

Tumors were fixed with 10% neutral buffered formalin, and embedded in paraffin according to standard histological procedures. H&E stained 5 μm thick sections were used to examine tumor morphology.

EXAMPLE 16

Histological Evaluation of 66 cl.4-GFP Cells

The murine mammary tumor cells were characterized as spindle cell carcinomas, poorly differentiated, with high mitototic index.

EXAMPLE 17

Statistical Analyses

Statistical analyses were conducted using Prism software version 3.0 (Graphpad, San Diego, CA). Animal numbers for experiments were determined by power calculation. Animal weights and tumor volumes were analyzed by student t-test.

EXAMPLE 18

Evaluation of anticancer properties of natural and synthetic tocopherols, tocotrienols and derivatives

Balb/c mice were injected with 200,000 66 cl.4 GFP cells subcutaneous in the mammary fat pad area between the 4th and 5th nipple on the right side of the body. 9 days after injection,

animals were split into groups (10 animals per group) with comparable size tumors ranging from 0.5 X 0.5 mm – 1 X 2 mm. Animals were treated with liposomal formulations of natural alpha and gamma tocopherols, natural alpha-tocotrienol, tocotrienol enriched fraction (TRF; this fraction contains approximately 32% alpha-tocopherol, 20% alpha tocotrienol, 31% gamma tocotrienol and 12% delta-tocotrienol; Gould, M. N. (30), synthetic dl-alpha-tocopherol, and synthetic derivative dl-alpha-tocopherol acetate by aerosol delivery for 19 or 21 days. Animals were given 75 mg of each compound via nebulization per day which delivered 36 μg of each compound to each animal per day. Animals were palpated every other day and volumes were calculated as $(w^2 \times l)/2$.

Data is shown only for RRR-gamma-tocopherol and dl-alpha-tocopherol (Figure 2). dl-alpha tocopherol liposomal formulation delivered by aerosol significantly inhibited the growth of 66 cl.4GFP cells by 81 and 73% on days 15 and 17 of treatment, respectively. Although not as effective as dl-alpha-tocopherol, dl-alpha-tocopherol acetate, RRR-delta-tocopherol, RRR-alpha-tocotrienol, tocotrienol rich fraction and (TRF), inhibited tumor growth by 29, 25, 34, 25, and at days 15 and by 57, 40, 27, and 40% at day 17, respectively.

RRR-α-tocopherol and RRR-γ-tocopherol liposomal preparations delivered by aerosol enhanced tumor growth. The enhancement of tumor growth, determined by tumor volume, by RRR-α-tocopherol was not significantly greater than tumor growth in controls; however, tumor volume of mice receiving

RRR-γ-tocopherol was significantly higher than the tumor volume of control mice (Figure 2).

Table 2 depicts the percent inhibition of visible lung metastases with these liposomal formulations of vitamin E or its derivatives. These data show that dl-a-tocopherol liposomal formulation delivered by aerosol significantly reduced the number of visible lung metastatic lesions in comparison to controls.

TABLE 2

Treatment	Visible lung metastases/ animal	Inhibition (%)
Control	1.7	0
dl-α-tocopherol	0.1	94**
dl-α tocopherol acetate	0.3	82
RRR-delta tocopherol	0.3	82
RRR-α tocopherol	1.7	0
RRR-γ tocopherol	1.15	32
RRR-α-tocotrienol	0.4	77
TRF	1.0	41

** significantly enhanced above controls

Immunohistochemical analyses of tumor sections from mice treated with dl-alpha-tocopherol liposomal formulation delivered by aerosol showed that this form of vitamin E is

inhibiting tumor growth by reducing tumor blood vessel numbers by 51%, inducing apoptosis by 44%, and reducing cell proliferation by 33%. Table 3 lists mechanisms whereby dl-alpha-tocopherol liposomal formulation delivered by aerosol inhibited growth of 66 cl.4-GFP tumors.

TABLE 3

Mechanisms of growth inhibition by dl-alpha-tocopherol liposomes

Treatment	CD31 Blood Vessel Nos/ Area	TUNEL Positive Apopotic Cells/ Area	KI67 Inhibition of Proliferation (%)
Control	227	1.4	60
dl-α-tocopherol	112	2.5	40
% Reduction	51%	----	33%
% Enhanement	---	44%	---

Furthermore, analyses of mechanisms of action of α-TEA in the transplantable, syngeneic mouse mammary cancer model show that α-TEA reduces cell proliferation and induces apoptosis. More specifically, mean values of the proliferation biomarker KI-67, detected by immunochemistry, were significantly reduced by 56% and mean apoptotic values, determined by TUNEL immunohistochemistry, were significantly increased by 30% in comparison to controls (unpublished data). Thus, it is contemplated that these surrogate markers may have

relevance as biomarkers for quantitatively and/or qualitatively determining chemoprevention efficacy of α-TEA.

EXAMPLE 19

VES and α-TEA Induce Apoptosis in 66 cl.4 GFP cells *In Vitro*

Previous studies indicate that vitamin E succinate is a potent apoptotic inducer in many human cancer cell lines, including breast cancer. For comparative purposes, we included vitamin E succinate in the *in vitro* analyses of α-TEA induced apoptosis. Balb/c mammary cancer 66 cl.4-GFP cells were treated with vitamin E succinate or α-TEA, and apoptosis was assessed by morphological analyses of DAPI stained cells for condensed nuclei and fragmented DNA.

Nuclei from 66 cl.4-GFP cells treated with 10 μg/ml α-TEA or vitamin E succinate for three days exhibited condensation and fragmented DNA, characteristics of apoptosis; whereas, nuclei from untreated cells did not exhibit these characteristics (Fig. 3A). 66 cl.4 GFP cells treated with 2.5, 5, 10, and 20 μg/ml α-TEA or vitamin E succinate for three days exhibited dose dependent apoptosis of 5, 6, 34, and 50 % apoptosis for α-TEA, and 3, 5, 16, and 34% apoptosis for vitamin E succinate (Fig. 2B). Untreated, VEH, and EtOH controls exhibited background levels of apoptosis of 2, 2, and 3%, respectively (Fig. 3B).

α-TEA was shown to induce apoptosis in a time-dependent manner. 66 cl.4 GFP cells treated with 10 μg/ml α-TEA for 2-5 days exhibited 20, 35, 47, and 58% apoptosis, respectively (Fig. 3C). Induction of apoptosis was confirmed by the presence

of PARP cleavage following treatment of 66 cl.4-GFP cells with 5, 10, and 20 μg/ml α-TEA for 48 hours (Fig. 3D). The 84 kDa cleavage fragment of PARP was evident at both 10 and 20 μg/ml α-TEA treatment; whereas, only intact PARP protein was detected in cells treated with 5 μg/ml α-TEA or in the untreated control cells (Fig. 3D).

EXAMPLE 20

Induction of Apoptosis by α-TEA *in Vivo*

In view of the *in vitro* data showing that α-TEA inhibits 66 cl.4-GFP tumor cell growth via induction of apoptosis, three tumors from each of the liposomal α-TEA/aerosol treatment and aerosol control groups were examined for apoptosis using TUNEL staining of 5 micron tumor sections. Tumors from mice treated with α-TEA had a mean ± S. E. of 2.04±0.23 apoptotic cells/field; whereas, tumors from aerosol control mice had a mean ± S.E. of 0.67±0.15 apoptotic cells/field ($p < 0.03$; Figure 4A). Positive stained apoptotic cells in tumor sections from liposomal α-TEA aerosol and control treated mice can be seen in Figure 4B.

EXAMPLE 21

α-TEA Inhibits 66 cl.4-GFP Clonal Growth

α-TEA at 1.25, 2.5, and 5 μg/ml decreased colony formation by 30, 85, and 100 % when compared to EtOH control (Fig. 5). Untreated (data not shown) and EtOH controls averaged 146 ± 11 S. D. and 140 ± 22 S. D. colonies, respectively. Cells

treated with α-TEA at 1.25 and 2.5 μg/ml averaged 98 ± 20 S. D. and 21 ± 6 S. D. colonies, respectively. No colonies formed when cells were treated with α-TEA at 5 (Fig. 5) or 10 μg/ml.

EXAMPLE 22

Effect of α-TEA liposomal aerosol treatment on body weight

Balb/c mice, 10/group (4 groups, 40 mice total) are inoculated with 200,000 66 cl.4 GFP cells as described on day 0. Nine days after tumor inoculation, treatments groups are initiated: Aerosol Treatment (TX) = aerosol delivery of α-TEA liposome (5 mg/mouse)/daily through day 23. Aerosol Control = Aerosol delivery of liposome composition only daily through day 23. Gavage treatment = gavage administration of α-TEA at 5 mg/mouse in ethanol/peanut oil daily through day 23. Gavage control = gavage administration of ethanol/peanut oil only through day 23. Mice were weighed at the initiation of treatment (day 9) and thereafter on days 13, 16, 20, and 23 (Fig. 6). Data are presented as the mean +/- standard deviation. There are no significant weight differences in the four groups.

EXAMPLE 23

Effect of α-TEA liposomal aerosol treatment on serum and tissue levels

Eight mice are treated via pulmonary aerosol delivery with 40mg of α-TEA (5 mg/mouse) in 6 ml liposomes. The mice inhaled the aerosol over a period of 30 minutes until delivery was complete. Mice are sacrificed 0, 2, 6, or 24 hours after the

treatment ended. Serum and tissue levels of α-TEA are determined by HPLC analyses (Fig. 7). At the first sacrifice (time 0), α-TEA is found only in the stomach tissue. α-TEA is present in the serum and stomach of mice sacrificed at 2 hours after completion of treatment. α-TEA is present in the liver and stomach at 6 hours after completion of treatment. α-TEA is present in the liver and stomach at 24 hours after completion of treatment.

EXAMPLE 24

Effect of α-TEA liposomal aerosol treatment on tumor weight

Mice are injected with 200,000 66cl.4 GFP murine mammary tumor cells as described on day 0. Treatments are initiated on day 9 when tumors reached 1-3 mm in size. Aerosol treatments of α-TEA liposomes (5 mg α-TEA /mouse) are administered daily for 16 days (Fig. 8). Data represent the mean +/- Standard Error (SE), N = 10 mice for control and treatment groups. At the time of sacrifice on days 25, 16 after treatment initiation, the size of tumors in the aerosol α-TEA liposomes treatment group are 61% less than the tumor size of the aerosol only control group.

EXAMPLE 25

Liposomal α-TEA/Aerosol Treatment Suppressed 66 cl.4-GFP Tumor Growth in Balb/c Mice and Reduced Lung Micrometastases

Balb/c mice were injected s. c. with 2 X 10^5 66 cl.4-GFP cells in the inguinal area between the 4th and 5th nipple on the

right side of the body. When tumors reached 2 x 2-4 x 4 mm (9 days after tumor injection), mice were placed into 5 groups ((Group 1: untreated control, Group 2: liposome/aerosol control, Group 3: liposomal α-TEA aerosol treatment, Group 4: peanut oil/gavage control, Group 5: α-TEA in peanut oil/gavage treatment) of 10 mice/group) such that the mean tumor volume of each group was closely matched. Daily treatments were initiated on day 9 after tumor injection.

The mean tumor volume of the liposomal α-TEA/aerosol treatment group, in comparison to aerosol control, was reduced by 23, 41, 50, 67 and 61% for days 9, 11, 13, 15, and 17 of treatment, respectively (Fig. 9A). At sacrifice, lungs were taken, examined visually for metastatic lesions and frozen for analyses of micrometastases by fluorescence. No visible tumors were seen in the α-TEA treatment group; whereas, the untreated and aerosol control groups exhibited 3.25±1.7 and 4.25±0.5 visual tumors/animals exhibiting lung metastases, respectively, as shown in Table 4.

Use of a Nikon fluorescence microscope and Image-Pro Plus software permitted measurement of green fluorescing micrometastases into three size groupings of <5 μm, 5-10 μm and >10 μm. This analysis showed a highly significant decrease in tumor metastases of all three sizes in the α-TEA treatment group in comparison to the aerosol and untreated controls (Fig. 9B). The mean number of micrometastases in the α-TEA treatment group (11.4 ± 3.5 S. E.; N= 8), in comparison to aerosol control (60.0 ± 15 S. E.; N = 10), was reduced by 81% ($p < 0.2$). Although the mean

number of micrometastases in the aerosol control group versus the untreated control (N = 10) was reduced (60±15.2 S. E. versus 101.7± 17.0 S. E.; p <0.9), the differences were not considered to be significant due to the great range in numbers of micrometastases among the mice within these two groups (Fig. 9B).

TABLE 4

66-cl.4-GFP Mammary Cancer Cell Lung Lung Metastases in Balb/c Mice

Treatments	No. Animals/Group with Visible Lung Metastases[a]	No. Visible Lung Tumor Foci/ Animal[b]
No Treatment	4/10	3.25±1.7
Aer./Liposome Control	4/10	4.25±0.5
Aer./Liposome/α-TEA	0/10	0

[a]Metastatic lesions in all five lung lobes for each animal in all treatment groups were counted visually at the time of sacrifice. [b]Data are expressed as the mean ±S.D. of visible lung tumor foci observed in the four lung metastases bearing animals in the two control groups.

EXAMPLE 26

Comparison of α-TEA and vitamin E succinate (VES) liposomes delivered via aerosolization on 66 cl.4 GFP tumor growth

Balb/c mice were injected with 200,000 66 cl.4 GFP cells subcutaneous in the mammary fat pad area between the 4th and 5th nipple on the right side of the body. 9 days after injection, animals were split into groups (10 animals per group) with comparable size tumors ranging from 0.5 X 0.5 mm – 1 X 2 mm. Animals were treated with α-TEA in liposome, vitamin E succinate in liposome, or control liposome alone via aerosol, 7 days per week, for 21 days. Animals were given 75 mg compound via nebulization per day such that each animal received 36 µg compound per day. Animals were palpated every other day and volumes were calculated as $(w^2 \times l)/2$. α-TEA liposome aerosol reduced tumor growth by 64, 76, 69, and 67% on days 15,17,19, and 21, respectively (values were statistically significantly different between α-TEA and control on days 17, 19, and 21, p= 0.03, 0.048, and 0.03, respectively). VES liposome aerosol reduced tumor growth by 76, 76, 69, and 68% on days 15,17,19, and 21, respectively (Fig. 10). Values were statistically significantly different between vitamin E succinate and control on days 17, and 21, p= 0.029, and 0.029 respectively.

EXAMPLE 27

Effect of gavage delivery of α-TEA on tumor weight

Delivery of α-TEA by gavage is ineffective in preventing the growth of 66 cl.4 GFP murine mammary tumor

cells at the inoculation site (Fig. 11). The size of the tumors, 9-21 days after injection of 200,000 66 cl.4 GFP cells between the 4th and 5th nipple on the right side of each mouse, is determined. Treatments are initiated on day 9 after tumor inoculation. Each group consists of n=10 +/- SE. Gavage Treatment = Treatments of 5 mg of α-TEA in ethanol and peanut oil (0.1 ml volume) are administered daily starting on day 9 after tumor inoculation and continuing through day 21. Gavage Control = Mice received peanut oil and ethanol alone (0.1ml volume)/daily, starting on day 9 after tumor inoculation and continuing through day 21. Data (tumor size) are depicted as the mean +/- standard error for days 9, 11, 13, 15, 17, 19, and 21. There are no significant differences in tumor weights between the control and gavage/α-TEA treatment groups at all time points.

EXAMPLE 28

Delivery of α-TEA by Gavage Did Not Reduce Tumor Growth at Inoculation Site But Reduced Lung Micrometastases

In contrast to liposomal α-TEA/aerosol treatment, mean tumor volumes from mice receiving 5 mg/day/mouse α-TEA EtoH/peanut oil formulation administered by gavage did not differ from the mean tumor volume of the gavage control (Fig. 12A). However, administration of α-TEA by gavage reduced the number of lung tumor micrometastases by 68%. The numbers of micrometastases, based on three size groupings (< 5 μm, 5-10 μm,

>10 μm), were 6.8±1.5, 11.3± 1.8 and 3.1±1.2 S. E. for mice administered α-TEA by gavage; whereas, micrometastases in control mice were 27.9±9.0, 29.2± 6.3, and 8.4±1.5 S. E., respectively (Fig. 12B).

No differences in mean body weights among any of the treatment or control groups were observed (data not shown). Non-tumor bearing mice that were treated with either aerosol/α-TEA or gavage for 17 days and then kept for eleven months to assess long term effects did not show any adverse effects of the α-TEA treatments.

Although administration of α-TEA by aerosol was superior to administration by gavage in that α-TEA administered by gavage did not reduce tumor size at the site of inoculation in comparison to tumor size of control mice, it is of interest that the number of lung micrometastases were reduced in comparison to control when α-TEA was administered by gavage. This suggests that α-TEA was bioavailable. Since α-TEA is non-hydrolyzable, and expectations are that when delivered by gavage, it should be an effective anti-tumor agent, it is contemplated that α-TEA administered at 5 mg/ml/day/mouse was not effective in reducing tumor growth at the site of tumor inoculation due to low uptake via the digestive tract. Thus, it is further contemplated that low levels of α-TEA may be effective in preventing lung tumor foci from being established.

EXAMPLE 29

Comparison of α-TEA/gavage, liposomal α-TEA/gavage and liposomal α-TEA/aerosol inhibition of tumor growth and lung and lymphnode metastases of 66 cl.4 mammary tumors

Although effective in inhibiting lung micrometastases, an α-TEA/EtOH/peanut oil formulation administered by gavage does not inhibit tumor growth in the transplantable syngeneic mammary cancer animal model. An α-TEA/liposomal formulation administered orally by gavage twice a day at 6 mg α-TEA/day, however, is an effective method of delivery for prevention of cancer growth as well as metastases of 66 cl.4 Balb/c mammary tumor cells in female Balb/c mice. The liposomal formulation of α-TEA delivered by gavage inhibits tumor growth by 70% and inhibits lung and lymphnode metastases by 59% and 56%. It is estimated that 36 μg of α-TEA is deposited in lungs of mice/day when α-TEA/liposomal preparations are administered by aerosol. The amount of α-TEA deposited in tissues when α-TEA/liposomal or α-TEA/EtOH/peanut oil formulations are given by gavage is not known. A comparison of anti-tumor efficacy of treatment regimes is shown in Table 5.

TABLE 5

Comparison of α-TEA Formulations and Methods of Administration In Prevention of Tumor Growth and Metastases of 66 cl.4 Balb/c mammary tumor cells

			Inhibition (%)		
	Delivery	α-TEA Conc	Tumor	Metastases	
Formulation	Method	(per mouse)	Growth	Lung	Lymphnode
Liposomal	Gavage	3 mg/2Xd	70%	59%	56%
Liposomal	Aerosol	5 mg/d	70%	81%	94%
EtOH/P Oil	Gavage	5 mg/d	0%	62%	not tested

EXAMPLE 30

Comparison of α-TEA and vitamin E succinate liposomes delivered via gavage on 66 cl.4 GFP tumor growth

α-TEA liposomal formulation delivered orally by gavage inhibited growth of murine mammary cancer cells transplanted into Balb/c mice. Vitamin E succinate liposomal formulation delivered orally by gavage did not inhibit growth of murine mammary cancer cells transplanted into Balb/c mice. Balb/c mice were injected with 200,000 66 cl.4 GFP cells subcutaneous in the mammary fat pad area between the 4th and 5th nipple on the right side of the body. 9 days after injection, animals were split into groups (10 animals per group) with

comparable size tumors ranging from 0.5 X 0.5 mm – 1 X 2 mm. Animals were treated with α-TEA in liposome, vitamin E succinate in liposome, or control liposome alone via oral gavage, 2 times per day, 7 days per week, for 21 days. Animals were given a total of 6 mg compound per day. Animals were palpated every other day and volumes were calculated as $(w^2 \times l)/2$. α-TEA liposome gavage reduced tumor growth by 85, 85, 80, and 70% on days 15,17,19, and 21, respectively (values were statistically significantly different between α-TEA and control on days 15, 17, and 19 ($p= 0.03$, 0.039, and 0.029, respectively). VES liposome gavage did not reduce tumor growth (Fig. 13).

EXAMPLE 31

Liposomal α-TEA gavage treatment reduced 66 cl.4-GFP lymphnode and lung micrometastases

Balb/c mice were injected with 200,000 66 cl.4 GFP cells subcutaneous in the mammary fat pad area between the 4th and 5th nipple on the right side of the body. 9 days after injection, animals were split into groups (10 animals per group) with comparable size tumors ranging from 0.5 X 0.5 mm – 1 X 2 mm. Animals were treated with α-TEA in liposome, vitamin E succinate in liposome, or control liposome alone via oral gavage, 2 times per day, 7 days per week, for 21 days. Animals were given a total of 6 mg compound per day. At sacrifice, the left lung lobe of each mouse of flash frozen and saves for detection of GFP expressing micrometatstic lesions via fluorescent microscopy.

Animals given α-TEA liposomal formulation via gavage, showed a marked decrease in micrometatstatic lung lesions compared to control (21.5/lung vs. 52.7/ lung, respectively). Vvitamin E succinate liposomal formulation **via gavage** had no effect on lung metastasis (Fig. 14A). At sacrifice, lymph nodes were collected and viewed for micrometastatic lung lesions. Animals given α-TEA via gavage showed lymph nodes with an average of 3.1 ± 0.8 miceo metastatic lesions vs 7.1 ± 1.7 micrometastatic lesions in the control group (p=0.036). Vitamin E succinate via gavage was not effective in decreasing micrometastatic lung lesions (Fig. 14B).

EXAMPLE 32

Liposomal α-TEA gavage treatment reduced tumor growth of MDA-MB-435 breast cancer cells *in vivo*

Nu/Nu athymic nude mice were injected with 1.0 X 10^6 MDA-MB-435 GFP FL cells subcutaneous in the mammary fat pad area between the 4^{th} and 5^{th} nipple on the right side of the body. 8 days after injection, animals were split into groups (10 animals per group) with comparable size tumors ranging from 0.5 X 0.5 mm – 2 X 2 mm. Animals were treated with alpha-TEA in liposome, or control liposome alone via oral gavage, 2 times per day, 7 days per week, for 21 days. Animals were given a total of 8 mg compound per day. Animals were palpated every other day and volumes were calculated as $(w^2 \times l)/2$. Alpha-TEA liposome gavage reduced tumor growth by 68, 75, 78, 79 and 79% on days

27, 29, 31, 33, and 35, respectively (Fig. 15). Values were statistically significantly different between a-TEA and control on days 33 and 35 (p= 0.045 and 0.033, respectively).

EXAMPLE 33

Inhibition of tumor growth and lung metastases of 66 cl.4-GFP tumors in Balb/c Mice by aerosol liposomal/α-TEA+9NC combination

Female Balb/c mice of 6 weeks of age were subcutaneously injected with 200,000 66 clone 4-GFP mammary tumor cells between the 4th and 5th nipples on the right side. When tumors reached 1 x 1 millimeters in size, aerosol treatments were initiated. α-TEA administered separately and in combination with low levels, 0.403 μg/day of 9-nitrocamptothecin is a potent inhibitor of tumor growth. Aerosol delivery of α-TEA + 9-nitrocamptothecin inhibited murine mammary tumor growth by 90%; whereas, α-TEA and 9-nitrocamptothecin alone inhibited tumor growth by 65% and 58%, respectively. Aerosol delivery of α-TEA + 9-nitrocamptothecin inhibited axillary lymph node and lung micrometastatic lesions by 87% and 71%; whereas, α-TEA and 9-nitrocamptothecin administered alone by aerosol inhibited lymph node micrometastatic lesions by 94% and 60%, and inhibited lung micrometastatic lesions by 71% and 55%, respectively. Thus, α-TEA + 9-nitrocamptothecin administered sequentially inhibits tumor growth better than when the two drugs are given separately. α-TEA separately is as effective in

inhibition of lung and lymphnode metastases as when administered in combination with 9-nitrocamptothecin. A comparison of data when α-TEA is administered separately and in combination with 9-nitrocamptothecin in the prevention of tumor growth and metastases is provided below in Table 6.

TABLE 6

Comparison of α-TEA Separately and In Combination with 9-Nitrocamptothecin (9NC) in Prevention of Tumor Growth and Metastases

			Inhibition (%)		
	Delivery	Drug Conc.	Tumor	Metastases	
Formulation	Method	In Nebulizer (per mouse)	Growth	Lung	Lymphnode
Lip/α-TEA	Aerosol	5 mg/d	65%	71%	94%
Lip/9NC	Aerosol	2 μg/d	58%	55%	60%
Lip/ α-TEA + 9-NC	Aerosol	5 mg+2μg/d	90%	71%	87%

EXAMPLE 34

α-TEA converts cisplatin resistant Cp-70 human ovarian cancer cells to cisplatin sensitive

Combinations of α-TEA + cisplatin enhances tumor cell killing *in vitro* and *in vivo*. The cp-70 cell line, a human ovarian

cancer, shows resistance to cisplatin, a platinum drug that is commonly used as a first-line treatment for ovarian tumors. The cp-70 sub-clone was developed *in vitro* from the A2780 cisplatin-sensitive cell line through intermittent exposure to increasing levels of cisplatin, up to 70 mM. When treated in culture with α-TEA, both cell lines A2780 and cp-70 undergo apoptosis (Fig. 16A). Treatment of cp-70 human ovarian cancer cells with sub-optimal levels of cisplatin (0.625 and 1.25 μg/ml) and α-TEA (10 μg/ml) restores cisplatin sensitivity to cp-70 cells, increases the levels of apoptosis in the A2780's and restores cisplatin-sensitivity in the cp-70 cell line (Fig. 16B). α-TEA in combination with cisplatin inhibits growth of cp-70 cells *in vivo*. The gene for green-fluorescent protein (GFP) was expressed in cp-70 cells via viral infection so that the cells would glow and could be used to study co-treatment effects in a xenograft mouse model. The combination treatment of cisplatin and α-TEA is tested in an *in vivo* xenograft nude mice model. In this model, female nude mice were inoculated with $1x10^6$ cells subcutaneously between the 4^{th} and 5^{th} nipples. When the average tumor size reached 1.0 mm^3, the mice were selected for treatment with either α-TEA alone, delivered via aerosol in liposomes, cisplatin alone (5mg/kg) injected I.P. once per week for 3 weeks, cisplatin + α-TEA, or aerosol control (liposome only). Data show α-TEA + cisplatin to be an effective method for inhibiting the growth of cisplatin resistant cp-70 human ovarian cancer cells (Fig. 16C).

EXAMPLE 35

Effect of alpha-TEA, methylselenocysteine and trans-resveratrol on MDA-MB-435-GFP-FL breast cancer cells

Female NU/NU homozygous mice were injected with one million MDA-MB-435 GFP FL cells subcutaneously in the mammary fat pad area between the 4th and 5th nipples on the right side of the body. Nine days after injection, animals were split into five groups of ten animals with comparable sized tumors ranging from 0.5mm x 0.5 mm to 2.0mm x 2.0mm. Animals were gavaged 3ppm methylselenocysteine (MSC) in water, α-TEA in liposome via aerosol, or gavaged 10 mg/kg b.w. *trans*-resveratrol (*t*-RES) in first 1:1, EtOH: physiological saline, and starting at day 24, 6.5% EtOH and 93.5% Neobee oil. A combination group was treated with each of the above three compounds, and a control group was treated with aerosolized liposome, 100 µl water, and 50 µl of the *t*-Res solvent. Animals were treated seven days a week, for 35+ days. Tumors were palpated every other day and volumes were calculated using the formula $(w^2 \times l)/2$.

At day 35, the α-TEA group showed a 47.8% decrease, the MSC group a 47.2% decrease, the *t*-RES group a 61.2% increase, and the combination group a 19.8% increase from the control (Fig. 17). The groups were statistically analyzed to the control with a t-test and the p values were p=0.0487, 0.0347, 0.2221, and 0.6255 for the α-TEA, MSC, *t*-RES, and combination group respectively. Thus, only the alpha-TEA and MSC groups tested separately gave reduced tumor growth significantly different from the controls.

Trans-resveratrol only treated and the combination of the three compounds enhanced tumor growth.

The following references are cited herein:

1. Prasad, et al., Effects of tocopherol (vitamin E) acid succinate on morphological alterations and growth inhibition in melanoma cells in culture. Cancer Res., *42*: 550-554, 1982.

2. Prasad, et al., Vitamin E and cancer prevention: Recent advances and future potentials. J. Am. Coll. Nutr., *11*: 487-500, 1992.

3. Schwartz, J., and Shklar, G. The selective cytotoxic effect of carotenoids and α-tocopherol on human cancer cell lines in vitro. J. Oral Maxillofac. Surg., *50*: 367-373, 1992.conserved homolog, Bax, that accelerates programmed cell death. Cell, *74*: 609-619, 1993.

4. Fariss, et al., The selective antiproliferative effects of α-tocopheryl hemisuccinate and cholesteryl hemisuccinate on murine leukemia cells result from the action of the intact compounds. Cancer Res., *54*: 3346-3351, 1994.

5. Kline, K., Yu, W., and Sanders, B. G. Vitamin E: Mechanisms of action as tumor cell growth inhibitors. *In*: K. N. Prasad and W. C. Cole (eds.), Proceeding of the International Conference on Nutrition and Cancer, pp 37-53. Amsterdam: IOS Press, 1998.

6. Kline, K., Yu, W , and Sanders, B. G. Vitamin E: Mechanisms of action as tumor cell growth inhibitors. J. Nutr., *131*: 161S-163S, 2001.

7. Neuzil, J., Weber, T., Gellert, N., and Weber, C. Selective cancer cell killing by α-tocopheryl succinate. Br. J. Cancer, *84*: 87-89, 2000.

8. Malafa, M.P., and Neitzel, L. T. Vitamin E succinate promotes breast cancer tumor dormancy. J. Surg. Res., *93*: 163-170, 2000.

9. Malafa, et al., Vitamin E inhibits melanoma growth in mice. Surgery, *131*: 85-91, 2002.

10. Neuzil, et al., Induction of cancer cell apoptosis by α-tocopheryl succinate: molecular pathways and structural requirements. FASEB J., *15*: 403-415, 2001.

11. Weber, et al., Vitamin E succinate is a potent novel antineoplastic agent with high selectivity and cooperativity with tumor necrosis factor-related apoptosis-inducing ligand (Apo2 ligand) in vivo. Clin. Cancer Res., *8*: 863-869, 2002.

12. Wu, et al., Inhibitory effects of RRR-alpha-tocopheryl succinate on benzo(a)pyrene (B(a)P)-induced forestomach carcinogenesis in female mice. World J. Gastroenterol., *7*: 60-65, 2001.

13. You, H., Yu, W., Sanders, B.G., and Kline, K. RRR-α-tocopheryl succinate induces MDA-MB-435 and MCF-7 human breast cancer cells to undergo differentiation. Cell Growth Differ., *12*: 471-480, 2001.

14. You, H., Yu, W., Munoz-Medellin, D., Brown, P. H., Sanders, B. G., and Kline, K. Role of extracellular signal-regulated kinase pathway in RRR-α-tocopheryl succinate-induced differentiation of human MDA-MB-435 breast cancer cells. Mol. Carcinogenesis, *33*: 228-236, 2002.

15. Yu, et al., Activation of extracellular signal-regulated kinase and c-Jun-NH2-terminal kinase but not p38 mitogen-activated protein kinases is required for RRR-α-tocopheryl succinate-induced apoptosis of human breast cancer cells. Cancer Res., *61*: 6569-6576, 2001.

16. Koshkina, et al., Distribution of camptothecin after delivery as a liposome aerosol or following intramuscular injection in mice. Cancer Chemother. Pharmacol., *44*: 187-192, 1999.

17. Knight, et al., Anticancer effect of 9-nitrocamptothecin liposome aerosol on human cancer xenografts in nude mice. Cancer Chemother. Pharmacol., *44*: 177-186, 1999.

18. Koshkina, et al., 9-Nitrocamptothecin liposome aerosol treatment of melanoma and osteosarcoma lung metastasis in mice. Clin. Cancer Res,. *6*: 2876-2880, 2000.

19. Walrep, et al., Pulmonary delivery of beclomethasone liposome aerosol in volunteers. Chest., *111*: 316-23, 1997.

20. Dexter, et al., Heterogeneity of tumor cells from a single mouse mammary tumor. Cancer Res., *38*: 3174-3181, 1978.

21. Miller, B. E., Roi, L. D., Howard, L. M., and Miller, F. R. Quantitative selectivity of contact-mediated intercellular communication in a metastatic mouse mammary tumor line. Cancer Res., *43*: 4102-4107, 1983.

22. Yu, et al., Vitamin E succinate (VES) induces Fas sensitivity in human breast cancer cells: role for Mr 43,000 Fas in VES-triggered apoptosis. Cancer Res., *59*: 953-961, 1999.

23. Tirmenstein, et al., Sensitive method for measuring tissue alpha-tocopherol and alpha-tocopheryloxybutyric acid by

high-performance liquid chromatography with fluorometric detection. J. Chromatogr. B Biomed. Sci. Appl., *707*: 308-311, 1998.

24. Schwenke, D. C. Does lack of tocopherols and tocotrienols put women at increased risk of breast cancer? J. Nutr. Biochem. *13*:2-20, 2002.

25. Kamal-Eldin, A. and Appelqvist, L.-A. The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids *31*:671-701, 1996.

26. Waldrep, et al., Operating characteristics of 18 different continuous-flow jet nebulizers with beclomethasone dipropionate liposome aerosol. Chest 105:106-110, 1994.

27. Waldrep, et al., Nebulized glucocorticoids in liposomes: Aerosol characteristics and human dose estimates. J Aerosol Medicine 7:135-145, 1994.

28. Pantazis, P. *et al.* Regression of human breast carcinoma tumors in immunodeficient mice treated with 9-nitrocamptothecin: differential response of nontumorigenic and tumorigenic human breast cells in vitro. Cancer Research 53:1577-82, 1993.

29. Chatterjee, D. *et al.* Induction of apoptosis in 9-nitrocamptothecin-treated DU145 human prostate carcinoma cells correlates with de novo synthesis of CD95 and CD95 ligand and down-regulation of cFLIP(short). Cancer Research 61:7148-7154, 2001.

30. Yu, W. *et al.* RRR-_-tocopheryl succinate-induction of DNA synthesis arrest of human MDA-MB-435 cells involves TGF-

α_ independent activation of p21 (Waf1/Cip1). Nutrition and Cancer In Press.

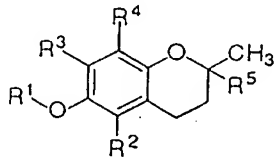
31. Gould, N. et al. A comparison of tocopherol and tocotrienol for the chemoprevention of chemically induced rat mammary tumors. Am. J. Clin. Nutr. 53: 1068S-1070S, 1991

Any patents or publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. Further, these patents and publications are incorporated by reference herein to the same extent as if each individual publication was specifically and individually incorporated by reference.

One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The present examples along with the methods, procedures, treatments, molecules, and specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention as defined by the scope of the claims.

WHAT IS CLAIMED IS:

1. A method for treating a cell proliferative disease comprising the step of administering a composition comprising a vitamin E based anti-cancer compound contained within a delivery vesicle to an individual in need of such treatment, said compound having a structural formula



wherein R^1 is a hydrogen or a carboxylic acid; R^2 and R^3 are hydrogen or R^4; R^4 is methyl; and R^5 is alkyl or alkenyl.

2. The method of claim 1, further comprising the step of administering a composition comprising an anticancer drug contained within a delivery vesicle.

3. The method of claim 2, wherein said anticancer drug/delivery vesicle composition is administered in combination with or sequentially with said vitamin E based anticancer compound/delivery vesicle composition.

4. The method of claim 3, wherein said anticancer drug/delivery vesicle composition is administered in combination with or sequentially with said vitamin E based anticancer compound/delivery vesicle composition, the delivery vesicle containing said vitamin E based anticancer compound also containing said anticancer drug.

5. The method of claim 2, wherein said anticancer drug is 9-nitrocamptothecin, cisplatin, paclitaxel, doxirubicin, or celecoxib.

6. The method of claim 1, wherein said vitamin E based anti-cancer compound is a tocopherol.

7. The method of claim 1, wherein said tocopherol is selected from the group consisting of β-tocopherol, δ-tocopherol, and 2,5,7,8-tetramethyl-(2R-(4R,8R,12-trimethyltridecyl) chroman-6-yloxy) acetic acid.

8. The method of claim 1, wherein said vitamin E based anti-cancer compound is a tocotrienol.

9. The method of claim 8, wherein said tocotrienol is selected from the group consisting of of α-tocotrienol, β-

tocotrienol, γ- tocotrienol, δ- tocotrienol, and tocotrienol enriched fraction.

10. The method of claim 1, wherein said vitamin E based anti-cancer compound is a synthetic vitamin E compound.

11. The method of claim 10, wherein said synthetic vitamin E compound is selected from the group consisting of dl-α-tocopherol, dl-α-tocopherol acetate, dl-α-tocopherol nicotinate, and dl-α-tocopherol phosphate.

12. The method of claim 1, wherein administration of said composition is via aerosol nebulization, an aerosol inhaler, gavage, oral ingestion, orally as a soft gel capsule, a transdermal patch, subcutaneous injection, intravenous injection, intramuscular injection, or intraperitoneal injection.

13. The method of claim 12, wherein aerosol nebulization of said composition is via a jet nebulizer.

14. The method of claim 1, wherein said delivery vesicle is a liposome comprising a lipid.

15. The method of claim 14, wherein said lipid is a 1,2-dilauroyl-sn-glycero-3-phosphocholine.

16. The method of claim 14, wherein a final concentration of said vitamin E based anti-cancer compound in said liposome is no greater than 20.0 mg/ml.

17. The method of claim 1, wherein said delivery vesicle is a nanoparticle, a microsphere or a niosome.

18. The method of claim 1, wherein said vitamin E based anti-cancer compound exhibits an anti-proliferative effect comprising apoptosis, DNA synthesis arrest, cell cycle arrest, or cellular differentiation.

19. The method of claim 18, wherein said anti-proliferative effect is determined via quantitative analysis, qualitative analysis or a combination thereof by detecting a biomarker or by a immunohistochemical assay.

20. The method of claim 19, wherein said biomarker is KI-67.

21. The method of claim 1, wherein said cell proliferative disease is selected from the group consisting of neoplastic diseases and non-neoplastic disorders.

22. The method of claim 21, wherein said neoplastic disease is selected from the group consisting of ovarian cancer, cervical cancer, endometrial cancer, bladder cancer, lung cancer, breast cancer, testicular cancer, prostate cancer, gliomas, fibrosarcomas, retinoblastomas, melanomas, soft tissue sarcomas, ostersarcomas, leukemias, colon cancer, carcinoma of the kidney, pancreatic cancer, basal cell carcinoma, and squamous cell carcinoma.

23. The method of claim 21, wherein said non-neoplastic disease is selected from the group consisting of psoriasis, benign proliferative skin diseases, ichthyosis, papilloma, restinosis, scleroderma, hemangioma, leukoplakia, viral diseases, and autoimmune diseases.

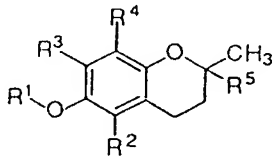
24. The method of claim 23, wherein said autoimmune diseases are selected from the group consisting of autoimmune thyroiditis, multiple sclerosis, myasthenia gravis, systemic lupus erythematosus, dermatitis herpetiformis, celiac disease, and rheumatoid arthritis.

25. The method of claim 21, wherein said non-neoplastic disorders are selected from the group consisting of viral disorders and autoimmune disorders.

26. The method of claim 25, wherein said viral disorder is Human Immunodeficiency Virus.

27. The method of claim 25, wherein said autoimmune disorders are selected from the group consisting of the inflammatory process involved in cardiovascular plaque formation, ultraviolet radiation induced skin damage and disorders involving an immune component.

28. A vesicle for delivery of a vitamin E based anticancer compound, said compound having a structural formula



wherein R^1 is a hydrogen or a carboxylic acid; R^2 and R^3 are hydrogen or R^4; R^4 is methyl; and R^5 is alkyl or alkenyl.

29. The vesicle of claim 28, wherein said vesicle is a liposome comprising a lipid.

30. The vesicle of claim 29, wherein the lipid comprising said liposome is 1,2-dilauroyl-sn-glycero-3-phosphocholine.

31. The vesicle of claim 25, wherein a ratio of vitamin E based anticancer compound to lipid is about 1:3 wt:wt.

32. The vesicle of claim 29, wherein said vesicle is a nanoparticle, a microsphere, or a niosome.

33. The vesicle of claim 28, wherein said vitamin E based anti-cancer compound is a tocopherol selected from the group consisting of α-tocopherol, β- tocopherol, γ-tocopherol, δ-tocopherol and 2,5,7,8-tetramethyl-(2R-(4R,8R,12-trimethyltridecyl) chroman-6-yloxy) acetic acid, a tocotrienol selected from the group consisting of α-tocotrienol, β- tocotrienol, γ- tocotrienol, δ- tocotrienol and tocotrienol enriched fraction, or a synthetic vitamin E compound selected from the group consisting of dl-α-tocopherol, dl-α-tocopherol acetate, dl-α-tocopherol nicotinate, and dl-α-tocopherol phosphate.

34. The vesicle of claim 33, wherein said vitamin E based anti-cancer compound is 2,5,7,8-tetramethyl-(2R-(4R,8R,12-trimethyltridecyl) chroman-6-yloxy) acetic acid.

35. The vesicle of claim 28, further comprising an anticancer drug.

36. The vesicle of claim 35, wherein said anticancer drug is 9-nitrocamptothecin, cisplatin, paclitaxel, doxirubicin, or celecoxib.

37. The vesicle of claim 28, wherein said vesicle delivers said vitamin E based compound via aerosol nebulization, an aerosol inhaler, gavage, oral ingestion, orally as a soft gel capsule, a transdermal patch, subcutaneous injection, intravenous injection, intramuscular injection, or intraperitoneal injection.

38. The vesicle of claim 37, wherein aerosol nebulization is via a jet nebulizer.

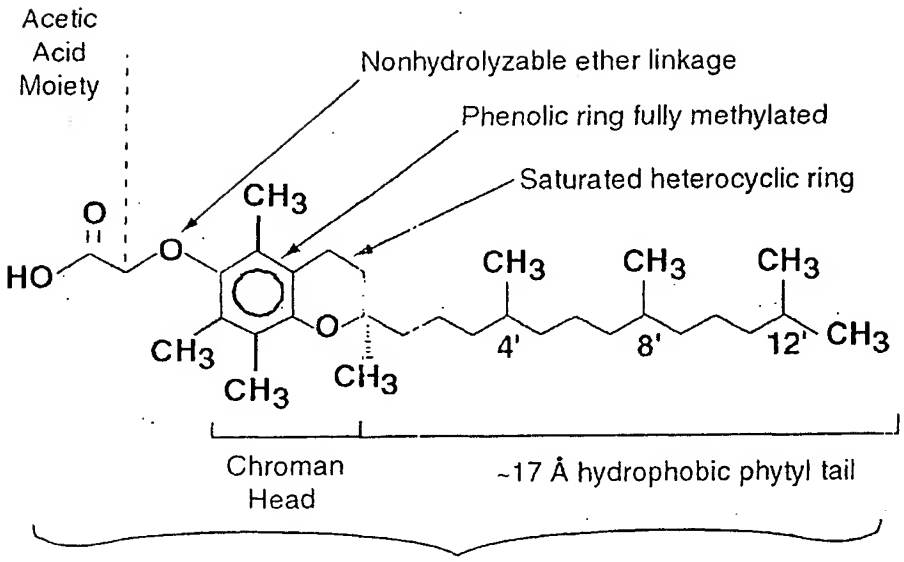

Acetic
Acid
Moiety
Nonhydrolyzable ether linkage
Phenolic ring fully methylated
Saturated heterocyclic ring
HO
O
O
CH3
CH3
CH3
CH3
CH3
CH3
CH3
4'
8'
12'
Chroman
Head
~17 Å hydrophobic phytyl tail
~29 Å in total length from H-bond donor to tip of side chain

Fig. 1

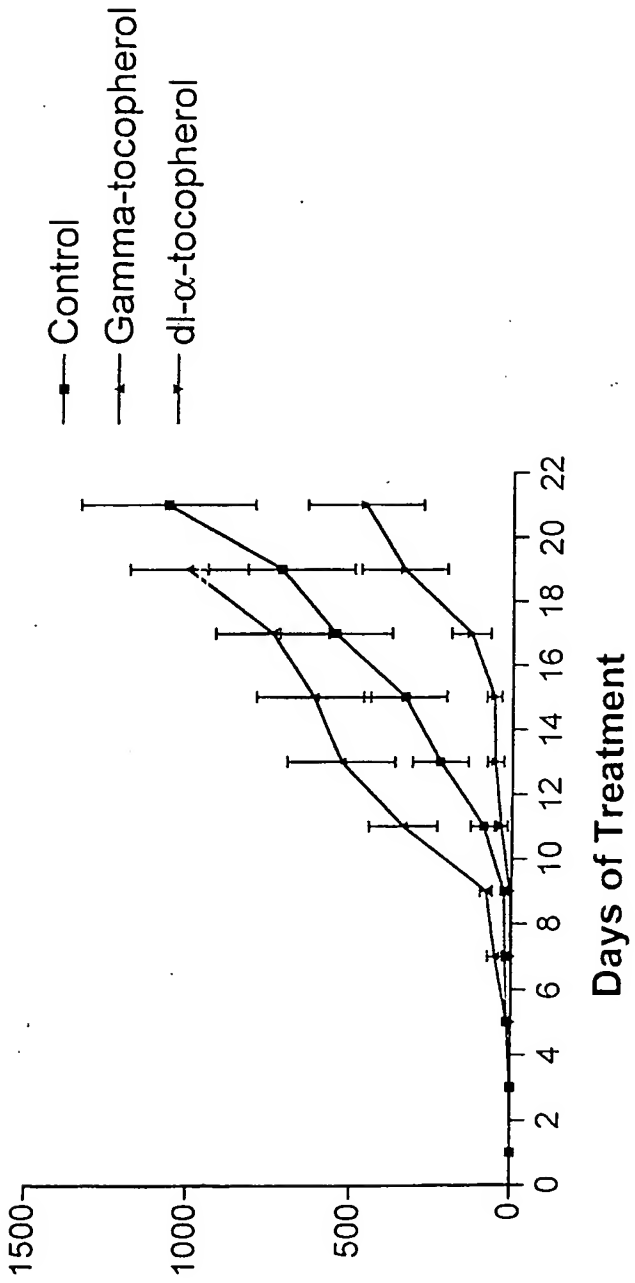

Control
Gamma-tocopherol
dl-α-tocopherol
1500
1000
500
0
0 2 4 6 8 10 12 14 16 18 20 22
Days of Treatment

Fig. 2

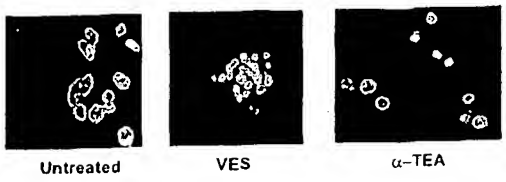


Fig. 3A

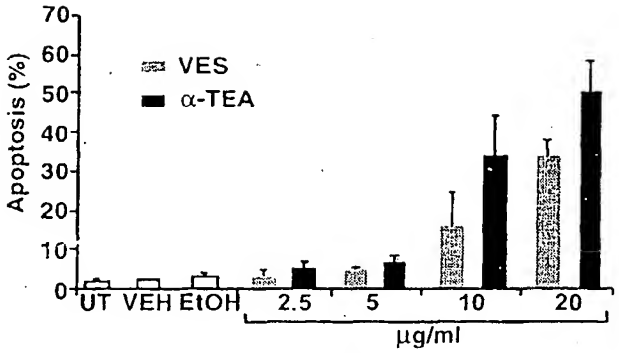


Fig. 3B

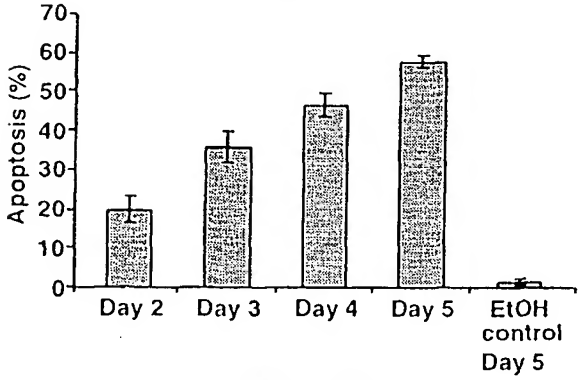


Fig. 3C

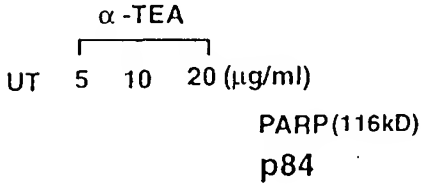


Fig. 3D

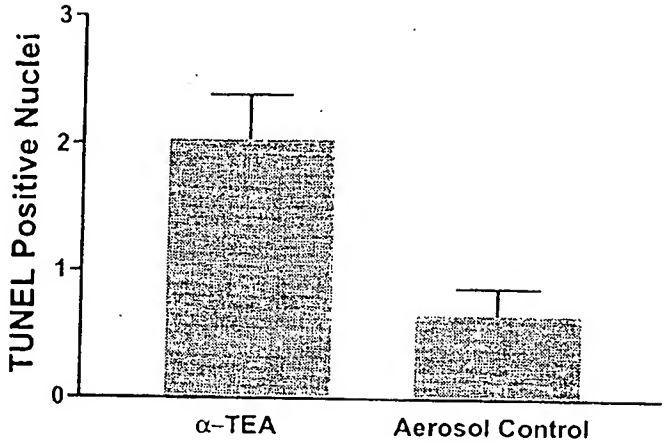

TUNEL Positive Nuclei
3
2
1
0
α-TEA
Aerosol Control

Fig. 4A

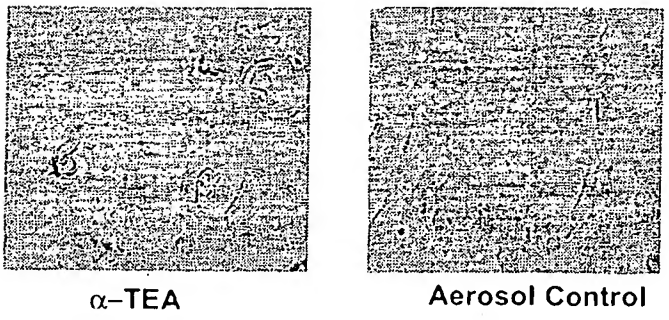
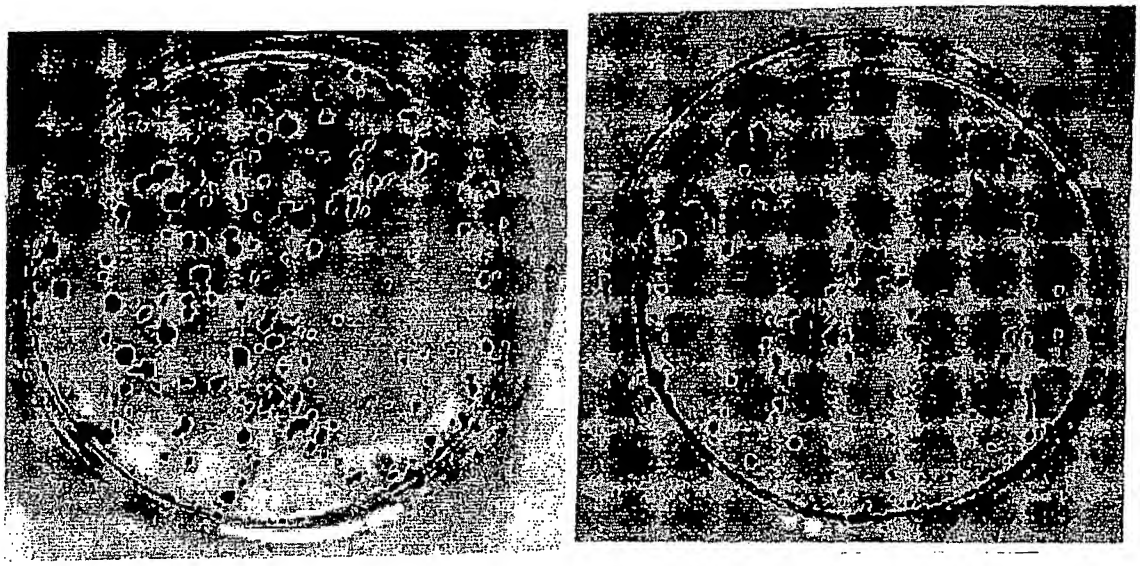
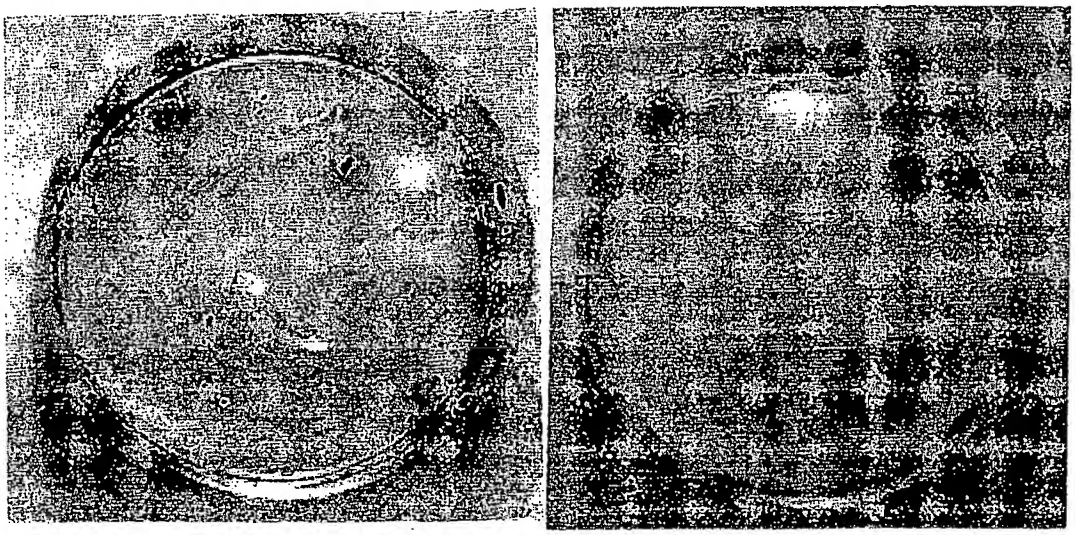

α-TEA
Aerosol Control

Fig. 4B



Control

1.25 μg/ml α-TEA
(2.55 μM)



2.5 μg/ml α-TEA
(5.1 μM)

5 μg/ml α-TEA
(10.2 μM)

Fig. 5

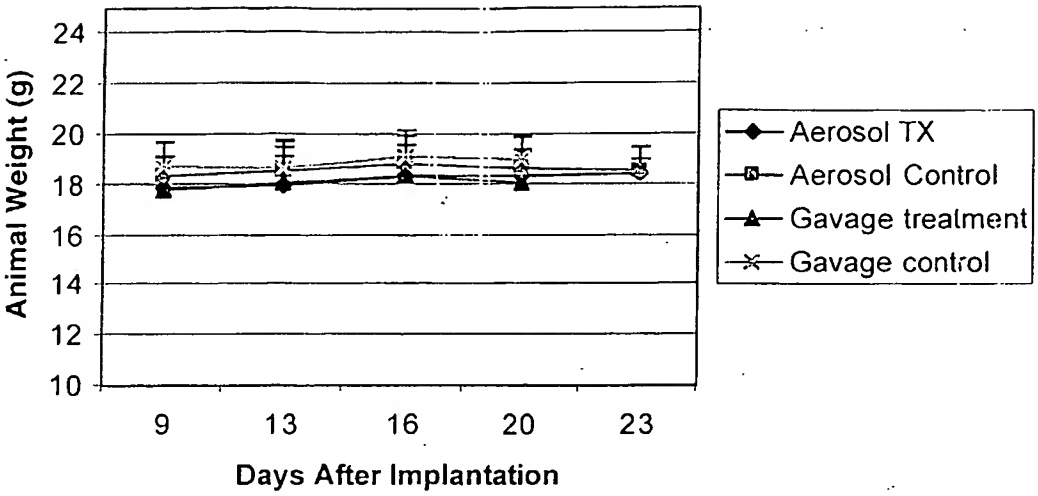

Animal Weight (g)
24
22
20
18
16
14
12
10
9
13
16
20
23
Days After Implantation
Aerosol TX
Aerosol Control
Gavage treatment
Gavage control

Fig. 6

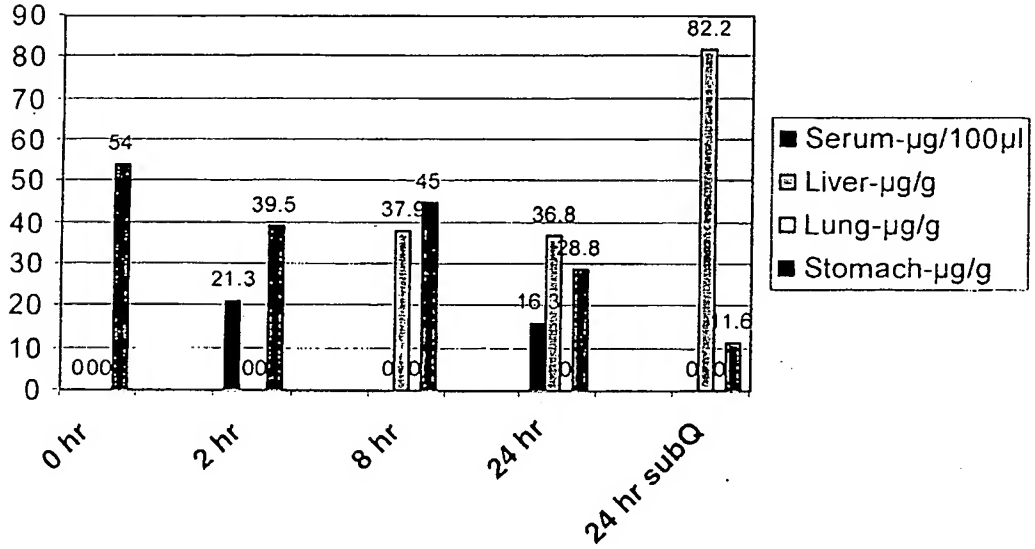

90
80
70
60
50
40
30
20
10
0
54
21.3
39.5
37.9
45
16
36.8
28.8
82.2
11.6
0 hr
2 hr
8 hr
24 hr
24 hr subQ
Serum-µg/100µl
Liver-µg/g
Lung-µg/g
Stomach-µg/g

Fig. 7

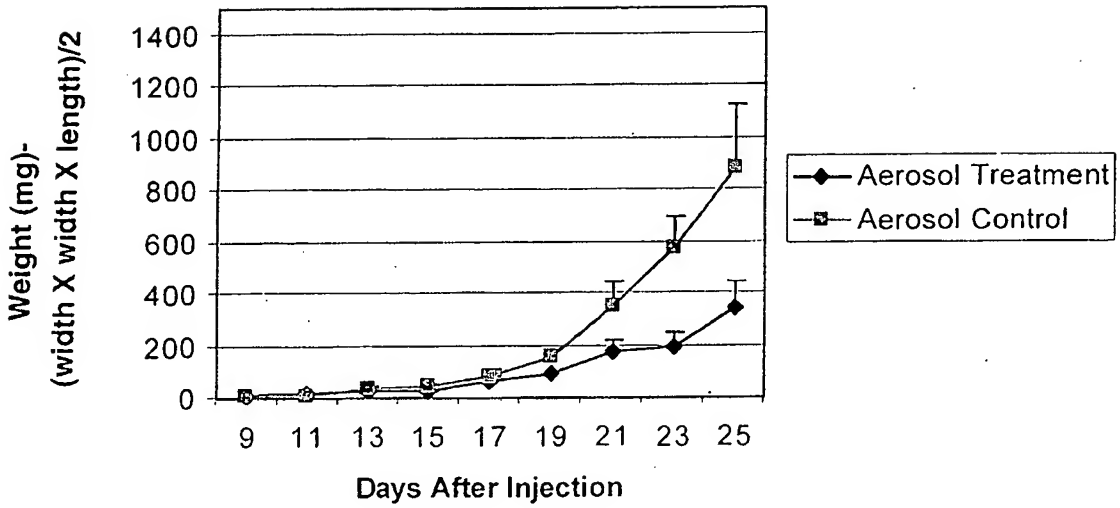

Weight (mg)-
(width X width X length)/2
1400
1200
1000
800
600
400
200
0
9
11
13
15
17
19
21
23
25
Days After Injection
Aerosol Treatment
Aerosol Control

Fig. 8

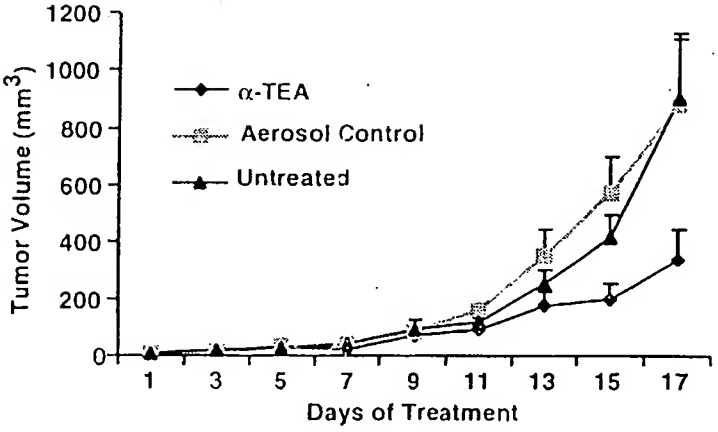

1200
1000
800
600
400
200
0
Tumor Volume (mm3)
α-TEA
Aerosol Control
Untreated
1
3
5
7
9
11
13
15
17
Days of Treatment

Fig. 9A

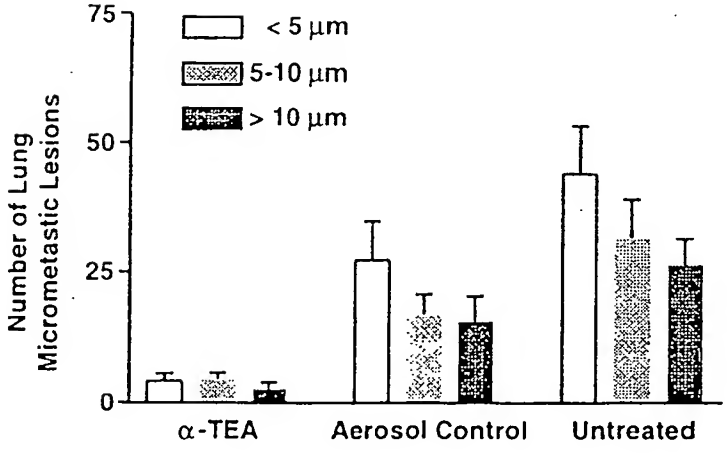

75
50
25
0
Number of Lung Micrometastic Lesions
< 5 µm
5-10 µm
> 10 µm
α-TEA
Aerosol Control
Untreated

Fig. 9B

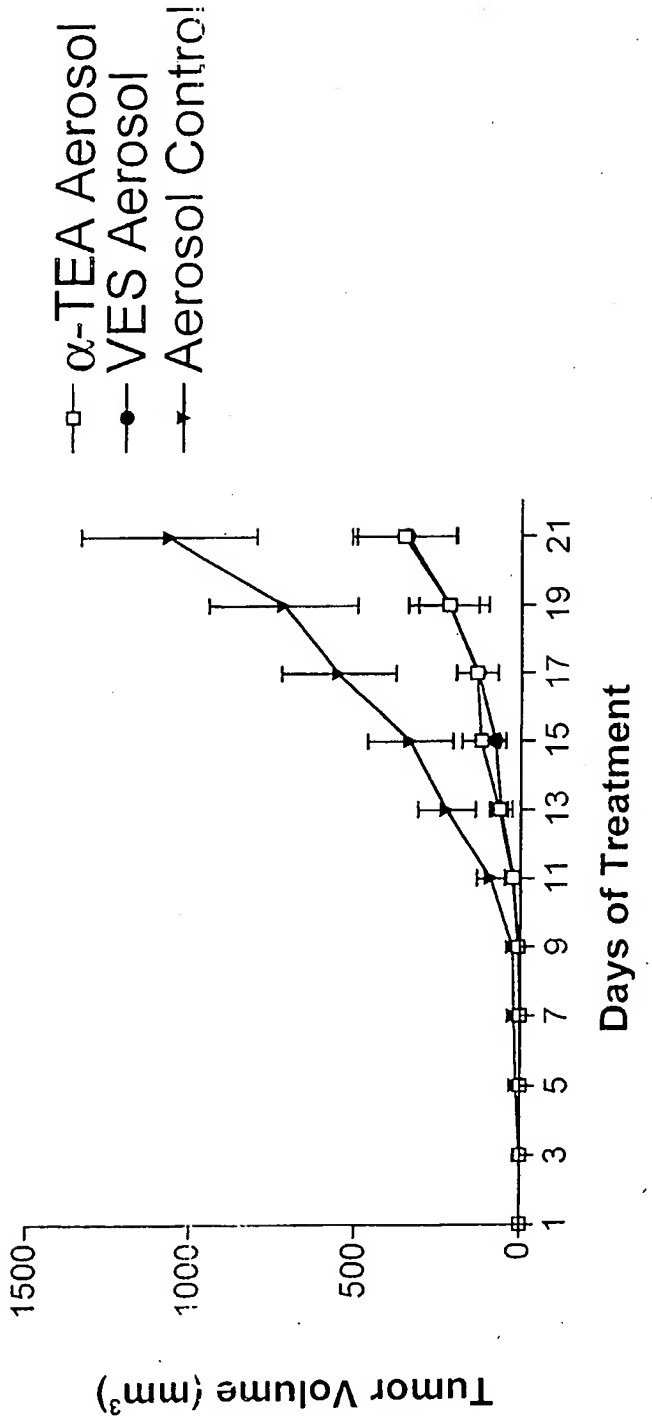

α-TEA Aerosol
VES Aerosol
Aerosol Control
Tumor Volume (mm³)
1500
1000
500
0
1
3
5
7
9
11
13
15
17
19
21
Days of Treatment

Fig. 10

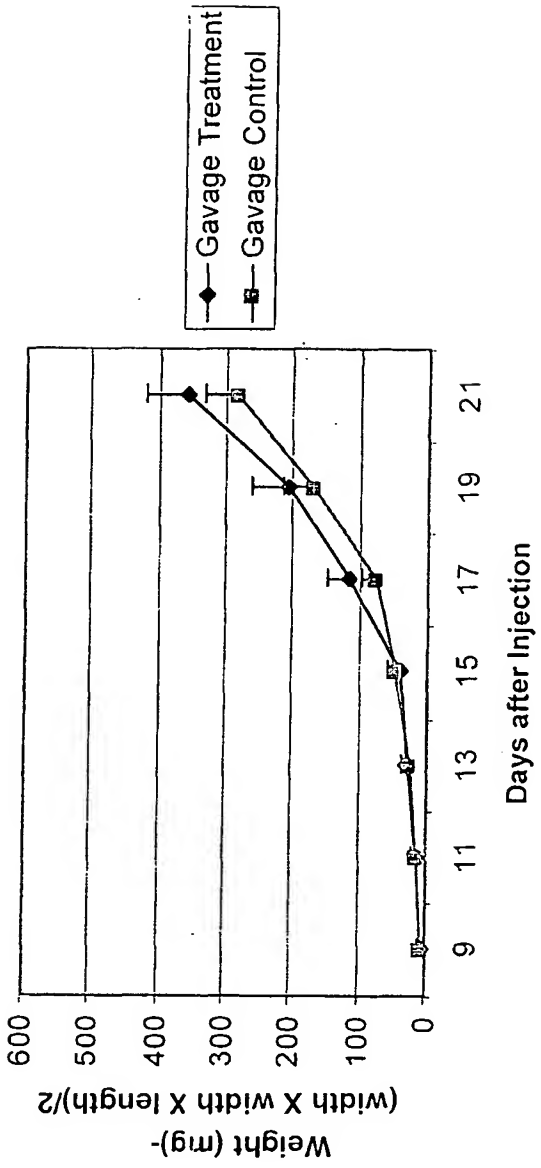

Gavage Treatment
Gavage Control
Weight (mg)-
(width X width X length)/2
600
500
400
300
200
100
0
9
11
13
15
17
19
21
Days after Injection

Fig. 11

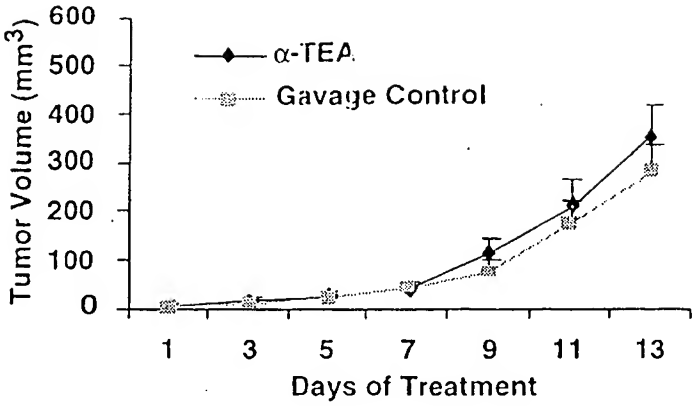


Fig. 12A

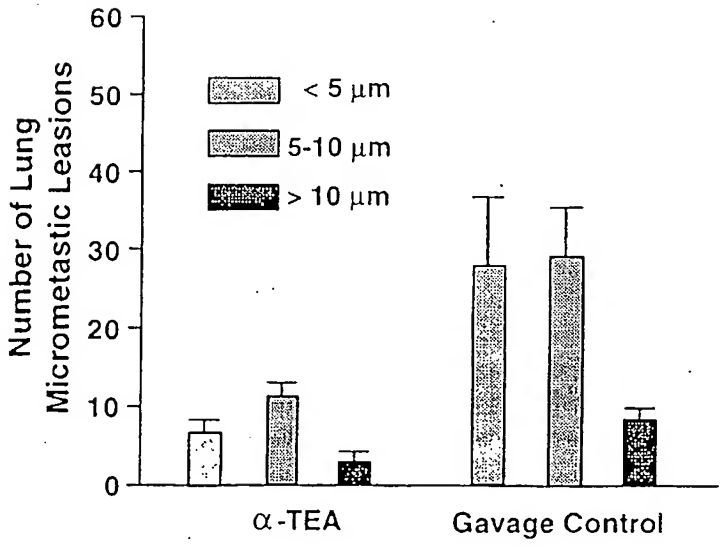


Fig. 12B

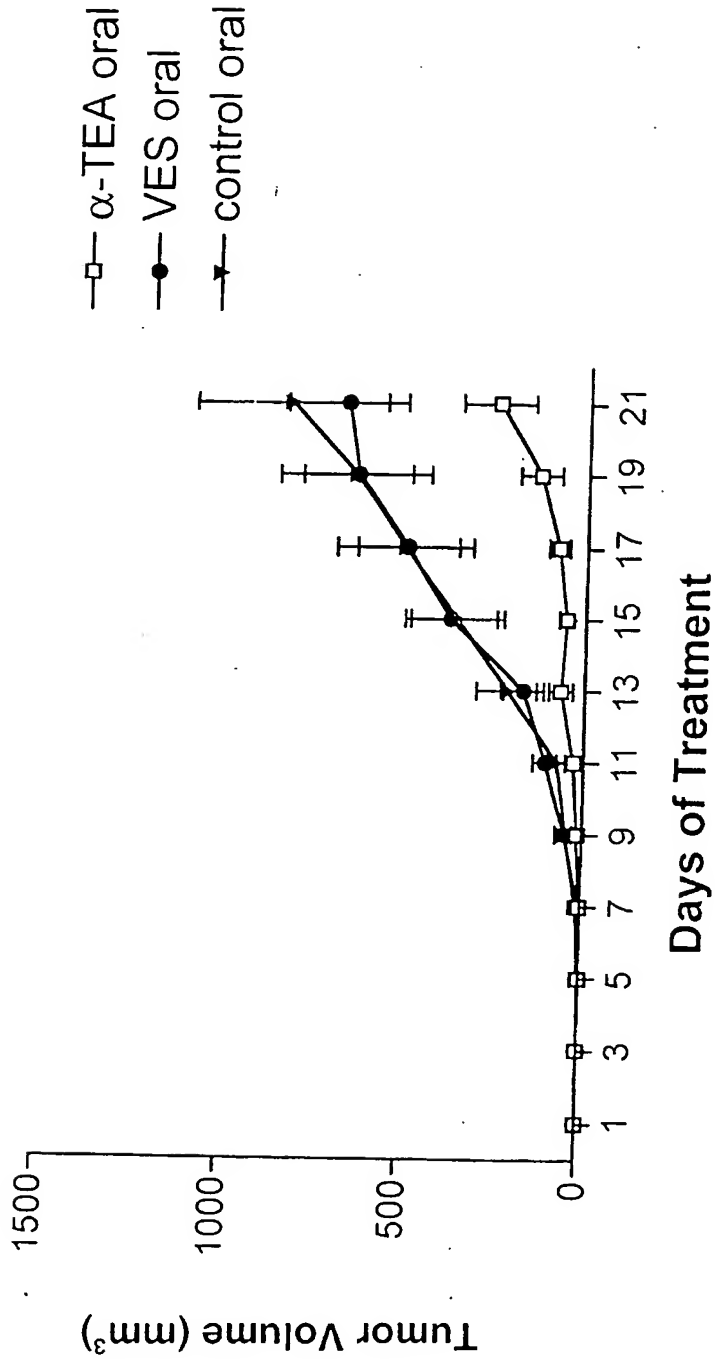

α-TEA oral
VES oral
control oral
Tumor Volume (mm³)
1500
1000
500
0
1
3
5
7
9
11
13
15
17
19
21
Days of Treatment

Fig. 13

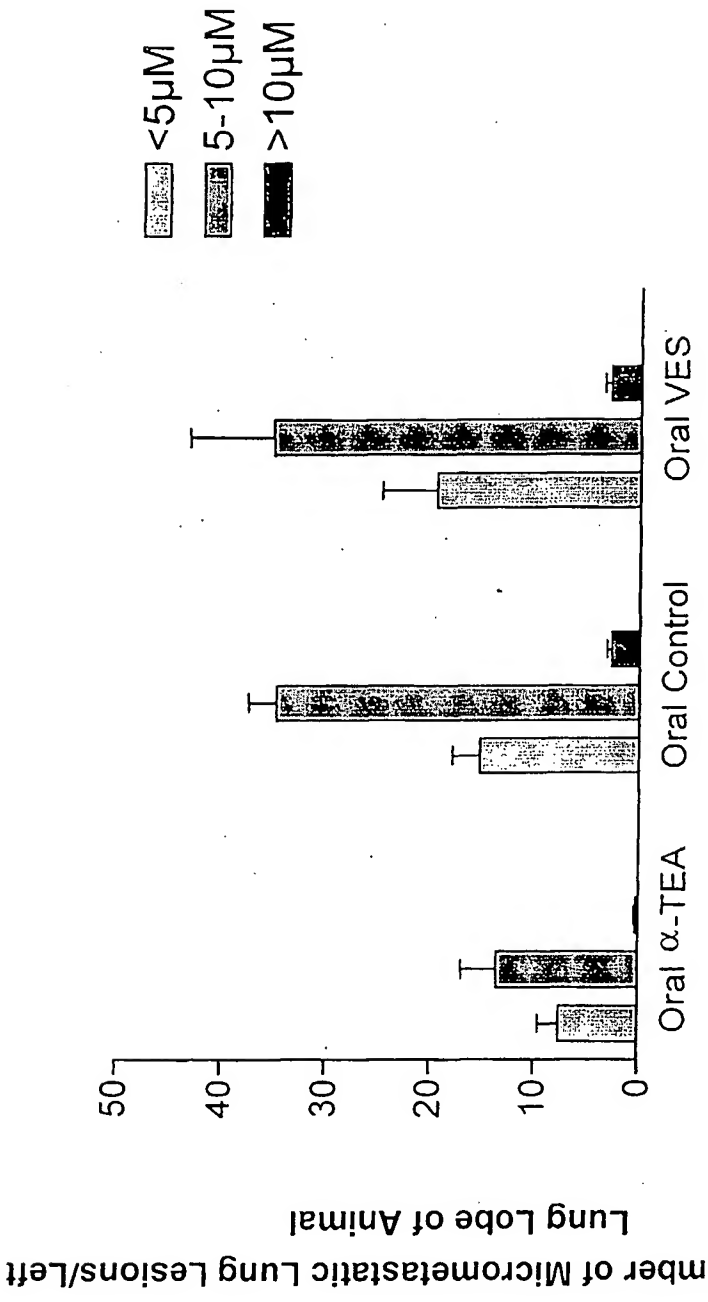

<5μM
5-10μM
>10μM
0
10
20
30
40
50
Oral α-TEA
Oral Control
Oral VES
Number of Micrometastatic Lung Lesions/Left Lung Lobe of Animal

Fig. 14A

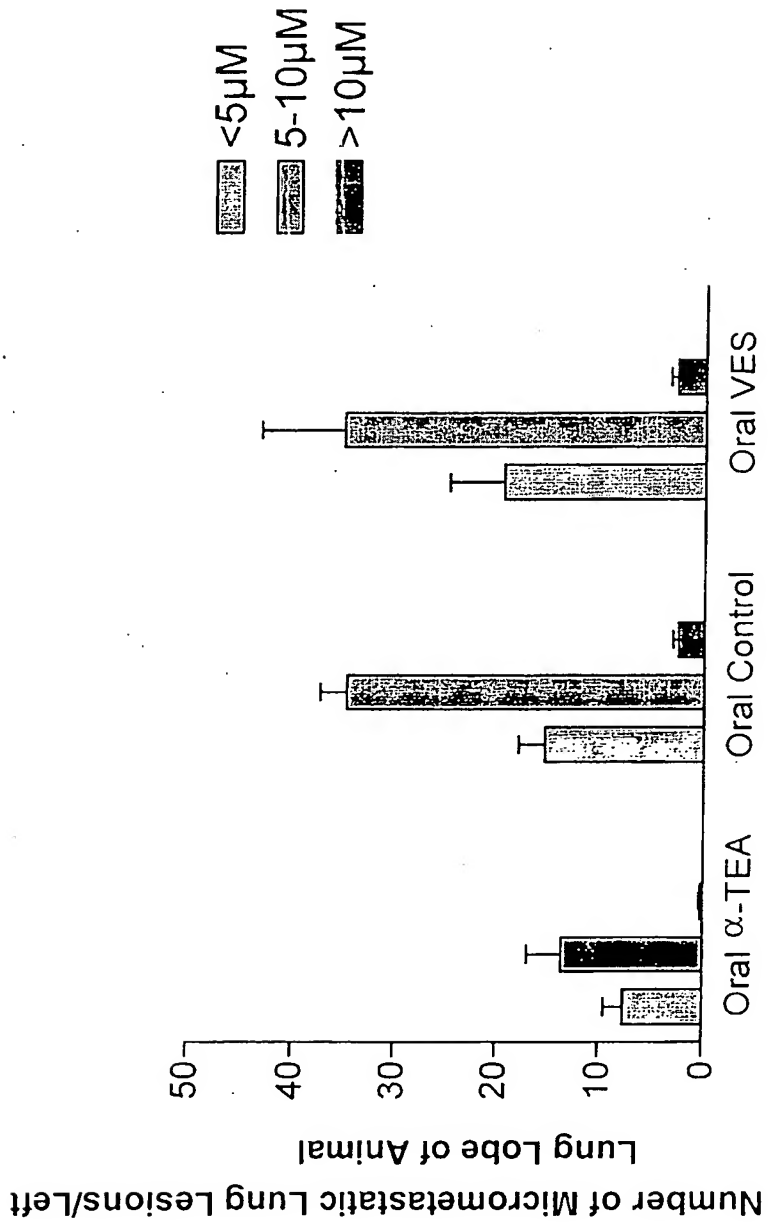

Number of Micrometastatic Lung Lesions/Left
Lung Lobe of Animal
0
10
20
30
40
50
Oral α-TEA
Oral Control
Oral VES
<5μM
5-10μM
>10μM

Fig. 14B

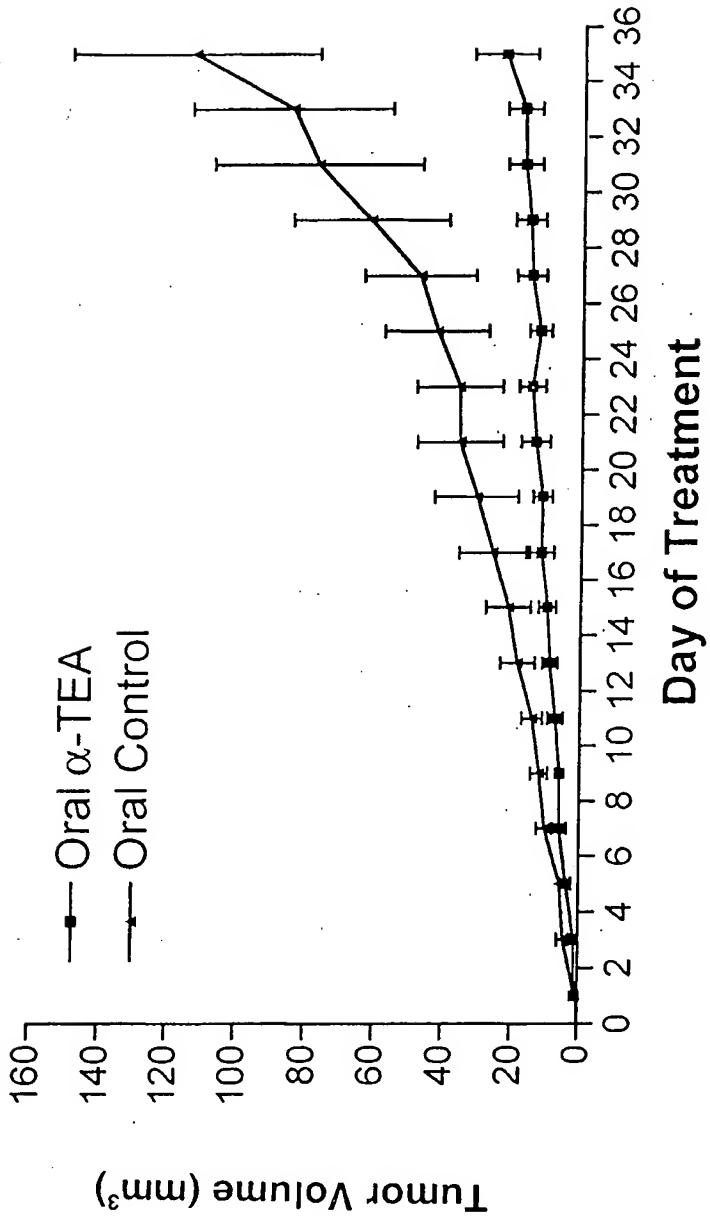

Oral α-TEA
Oral Control
Tumor Volume (mm³)
Day of Treatment
0
20
40
60
80
100
120
140
160
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36

Fig. 15

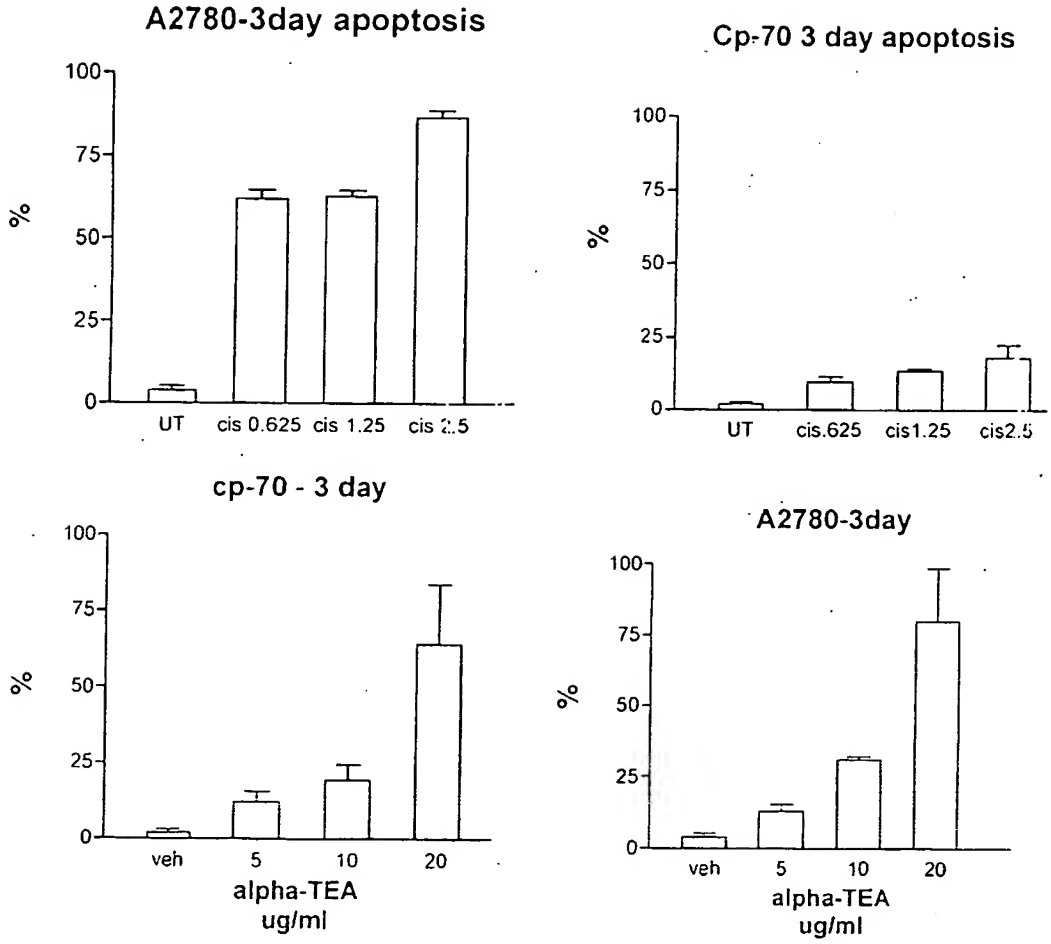
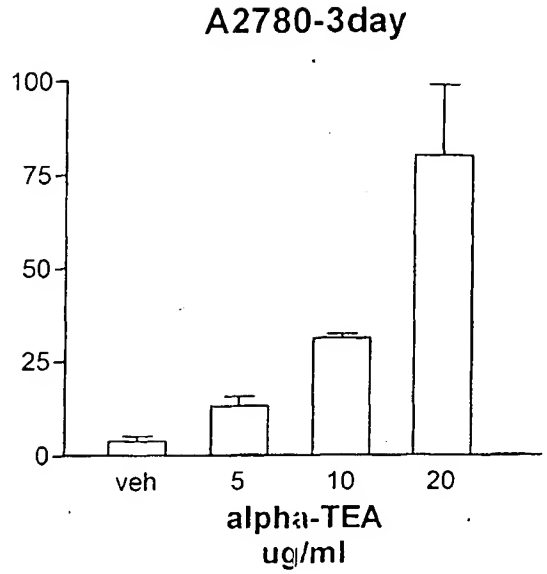

A2780-3day apoptosis
100
75
50
25
0
%
UT
cis 0.625
cis 1.25
cis 2.5
Cp-70 3 day apoptosis
100
75
50
25
0
%
UT
cis.625
cis1.25
cis2.5
cp-70 - 3 day
100
75
50
25
0
%
veh
5
10
20
alpha-TEA
ug/ml
A2780-3day
100
75
50
25
0
%
veh
5
10
20
alpha-TEA
ug/ml

Fig. 16A


A2780-3day
100
75
50
25
0
veh
5
10
20
alpha-TEA
ug/ml

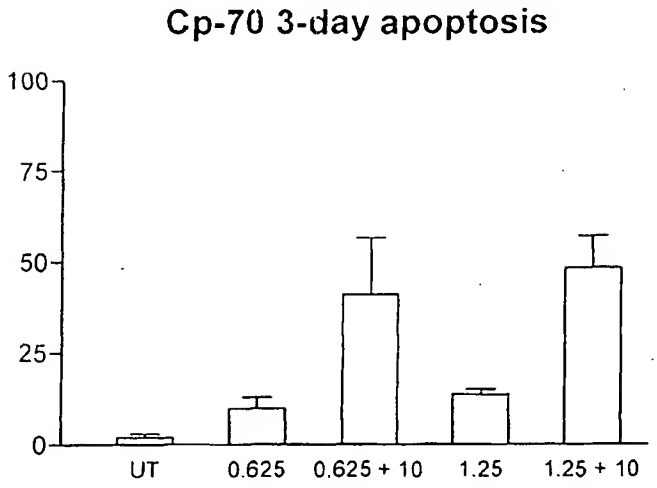

Cp-70 3-day apoptosis
100
75
50
25
0
UT
0.625
0.625 + 10
1.25
1.25 + 10

Fig. 16B

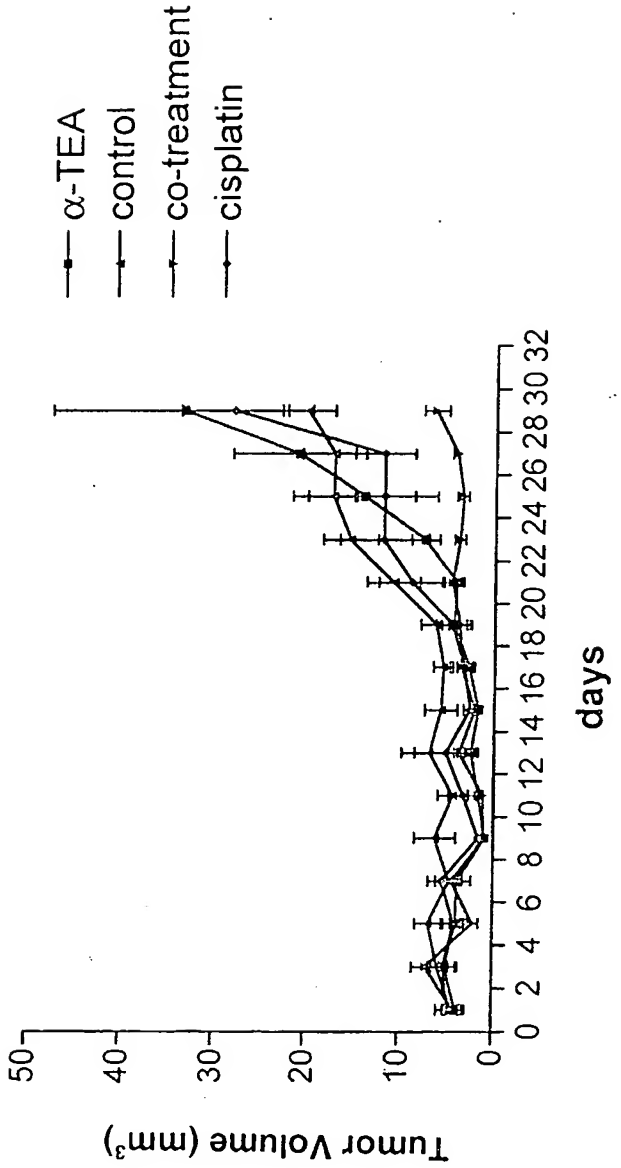

α-TEA
control
co-treatment
cisplatin
Tumor Volume (mm³)
0
10
20
30
40
50
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
days

Fig. 16C

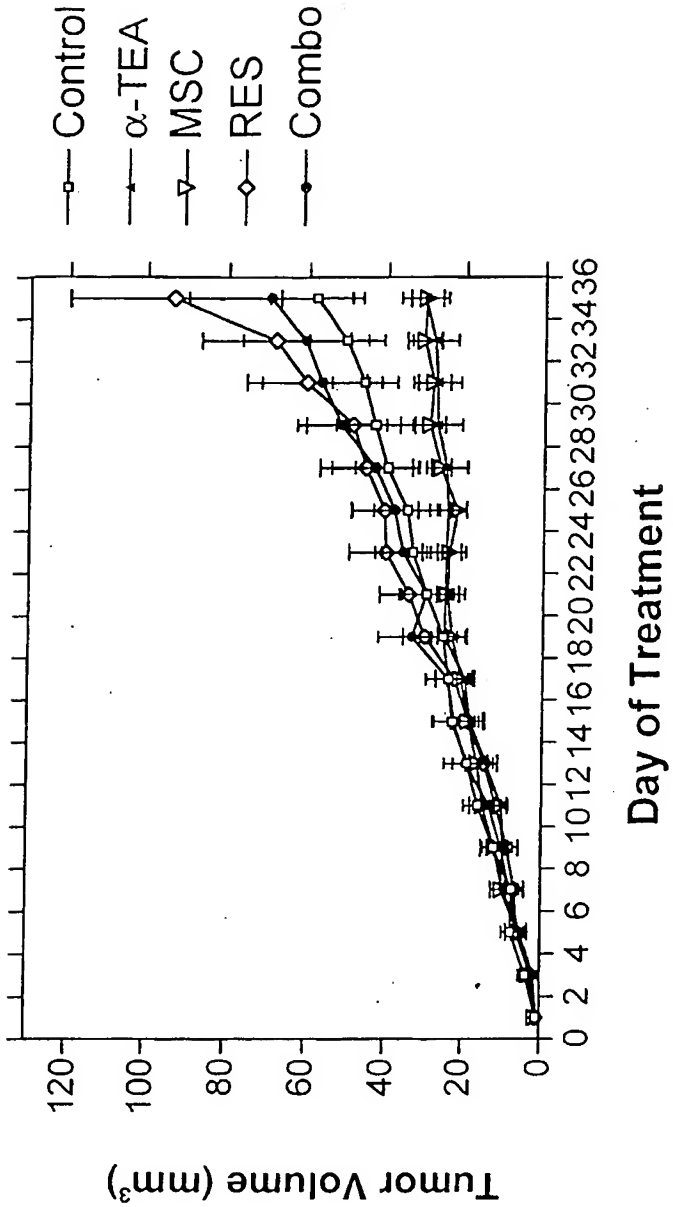

Control
α-TEA
MSC
RES
Combo
Tumor Volume (mm³)
120
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
Day of Treatment

Fig. 17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/40846

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :A61K 9/127, 50, 51
US CL : 424/450, 489; 514/458
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/450, 489; 514/458

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,873,088 A (MAYHEW et al) 10 October 1989, abstract, col. 4, line 59 through col. 8, line 22, col. 11, line 50 through col. 12, line 28, Examples and claims.	1-6, 10-12, 16-19, 21-22, 28-29, 31-33 & 35-37
Y	US 5,997,899 A (YE et al) 07 December 1999, abstract, col. 5, lines 3-5 and claims.	15 & 30
Y	US 4,181,725 A (VOORHEES et al) 01 January 1980, claim 1.	21 & 23
Y	US 5,114,957 A (HENDLER et al) 19 May 1992, abstract, Examples and claims.	25 & 26
Y	US 5,403,834 A (MALFROY-CAMINE et al) 04 April 1995, col. 9, lines 14-28.	22-25

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 08 APRIL 2003	Date of mailing of the international search report 07 MAY 2003
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer GOLLAMUDI S RISHORE Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/40846

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,895,719 A (RADHAKRISHNAN et al) 23 January 1990, abstract and claims.	13 & 38

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/40846

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/40846

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

WEST:
Search terms: liposomes, nanospheres, microspheres, niosomes, tocopherol, tocotrienls, cancer, neoplastic, neoplasia, cisplatin, paclitaxel. doxorubicin, celecoxib, camptothecin.